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(54) **PLANT PRODUCING HYALURONIC ACID**

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435/468; 536/23.1; 536/23.2; 536/23.7

(58) **Field of Classification Search** None
See application file for complete search history.

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(57) **ABSTRACT**

It is intended to provide by improving a known method of producing hyaluronic acid in a plant, a plant or a cultured plant cells which can produce hyaluronic acid at a lower cost and a further higher yield than before, a method of preparing the same, an expression vector for transformation, a method of producing hyaluronic acid using the plant or the cultured plant cells and the like. The method of producing hyaluronic acid comprising obtaining hyaluronic acid by co-expressing a protein with hyaluronic acid synthase activity and an exogenous protein with sugar-nucleotide synthase activity in a plant cell or a plant is provided.

38 Claims, 6 Drawing Sheets

FIG. 1

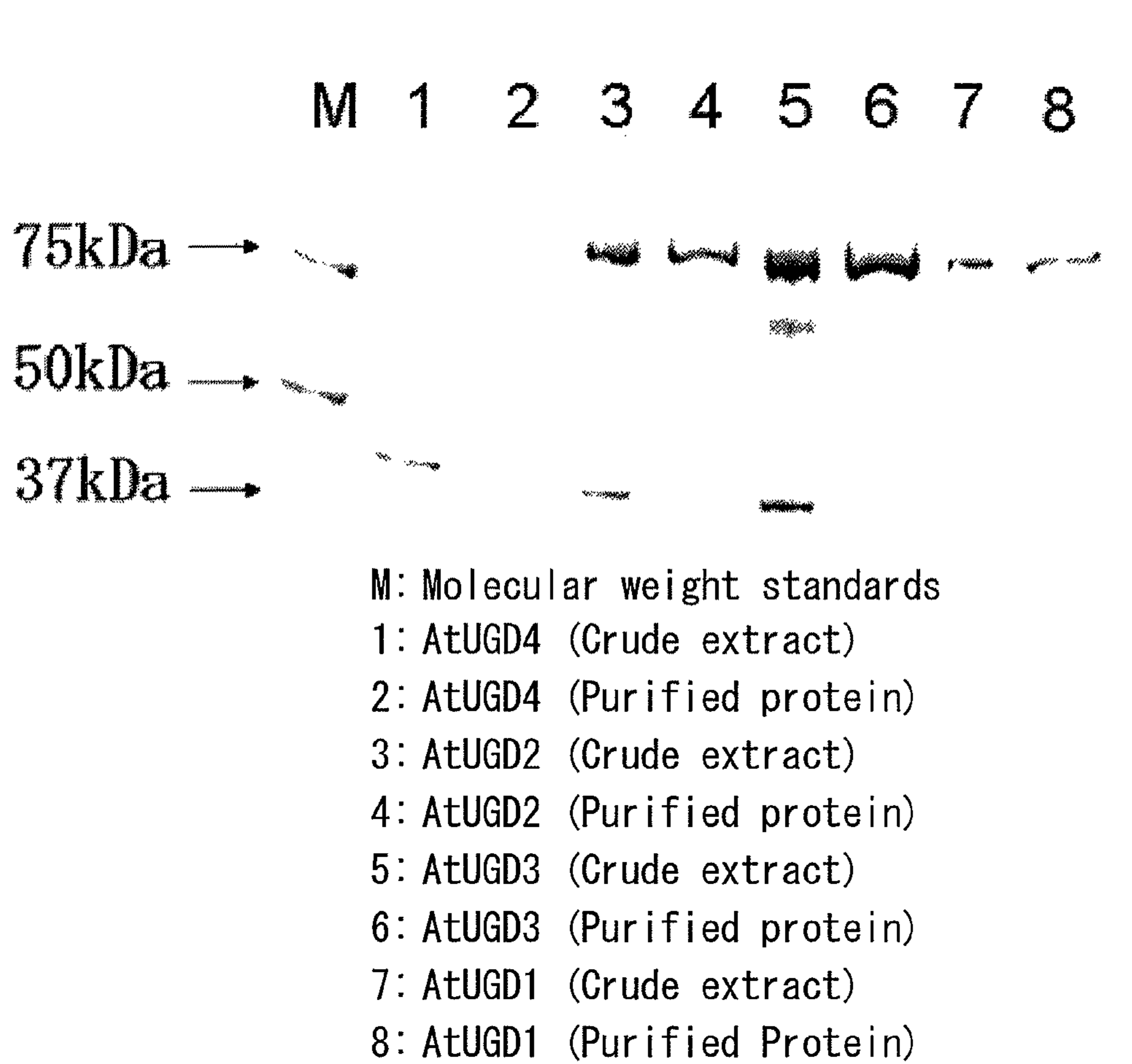
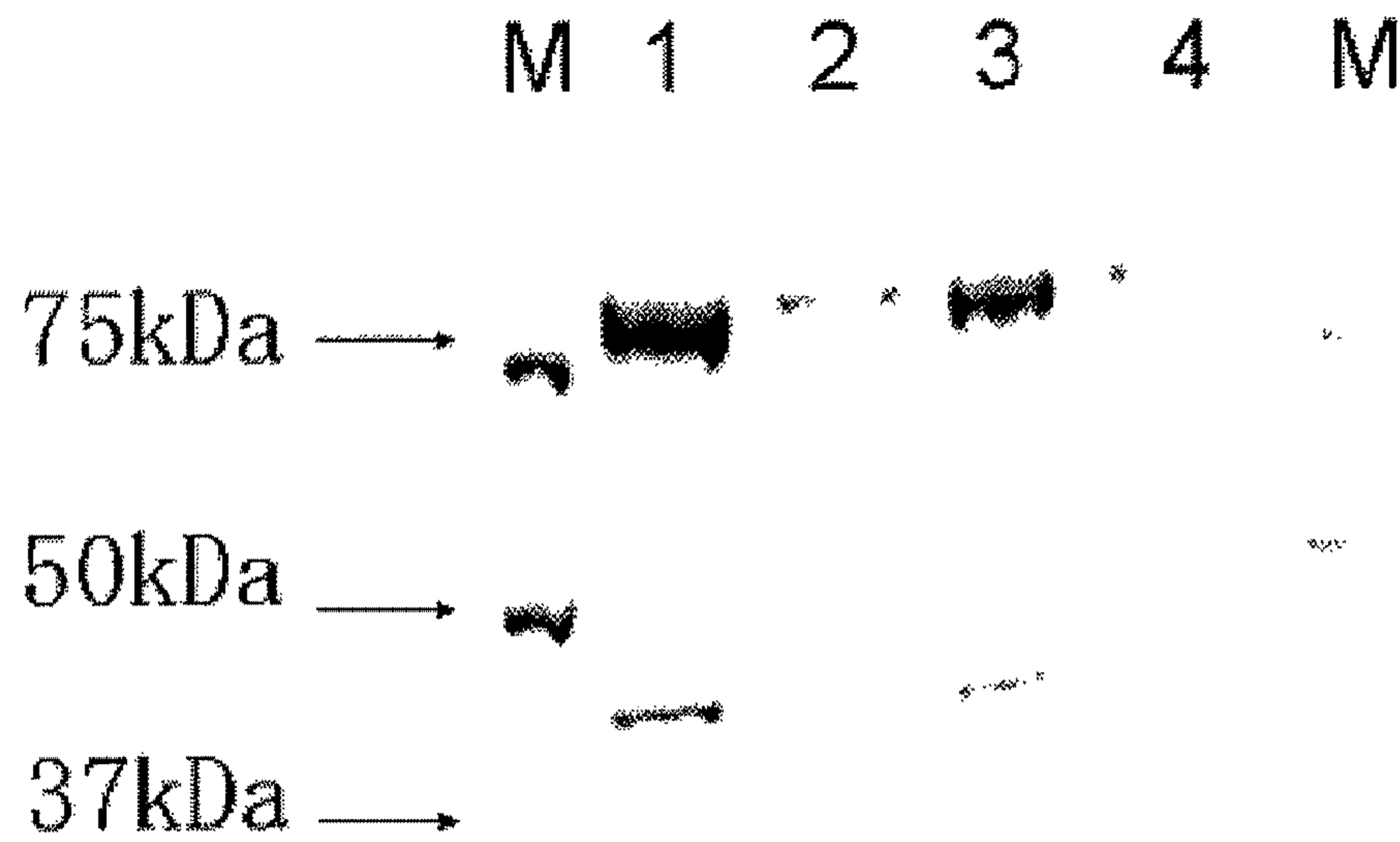
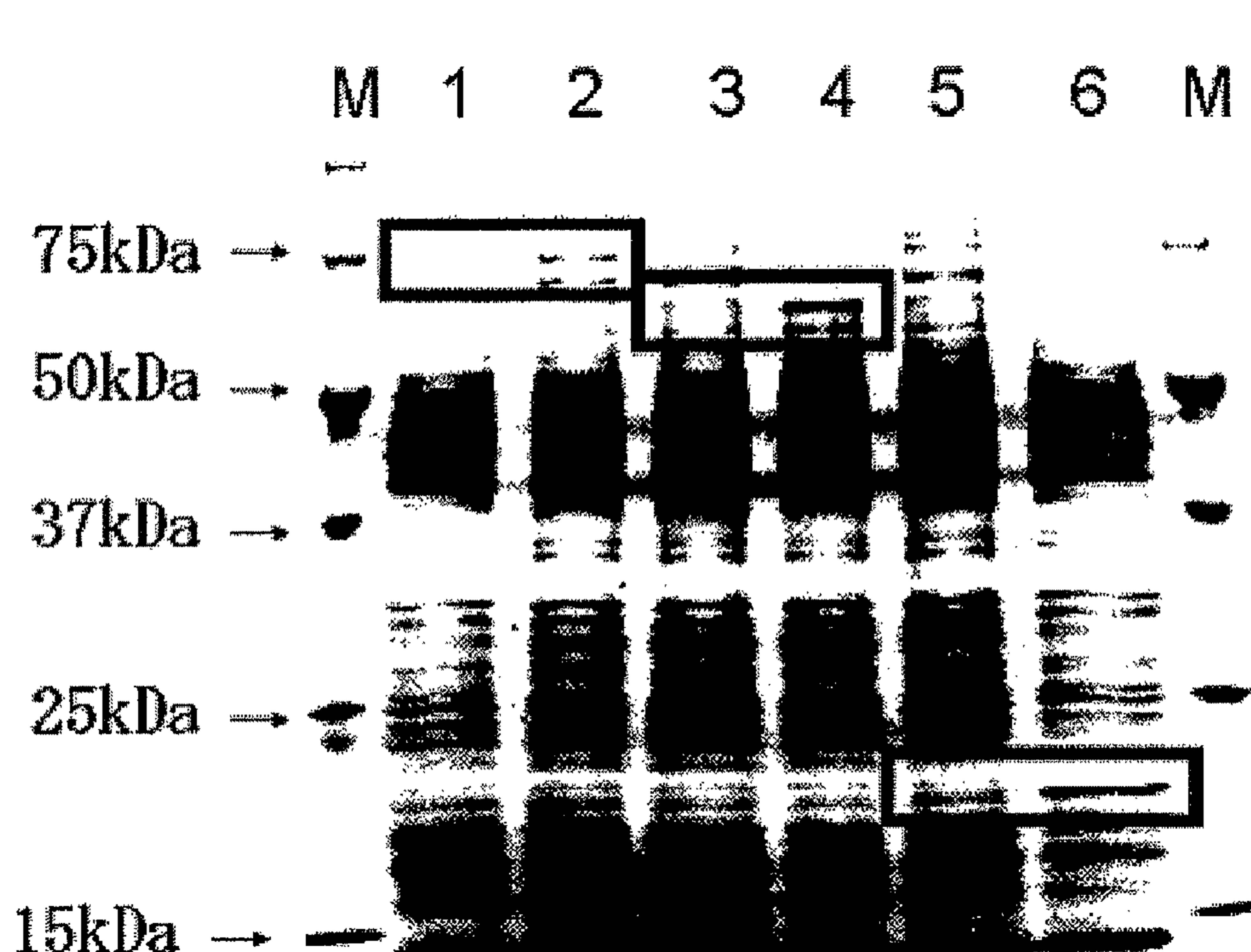


FIG. 2



M: Molecular weight standards
1: cvUGD-HI (Crude extract)
2: cvUGD-HI (Purified protein)
3: cvUGD-KA (Crude extract)
4: cvUGD-KA (Purified protein)

FIG. 3



M: Molecular weight standards
 1: AtGFAT (Before Reaction)
 2: AtGFAT (After 24-hour Reaction)
 3: cvGFAT-HI (Before Reaction)
 4: cvGFAT-HI (After 24-hour Reaction)
 5: DHFR (Before Reaction)
 6: DHFR (After 24-hour Reaction)

FIG. 4

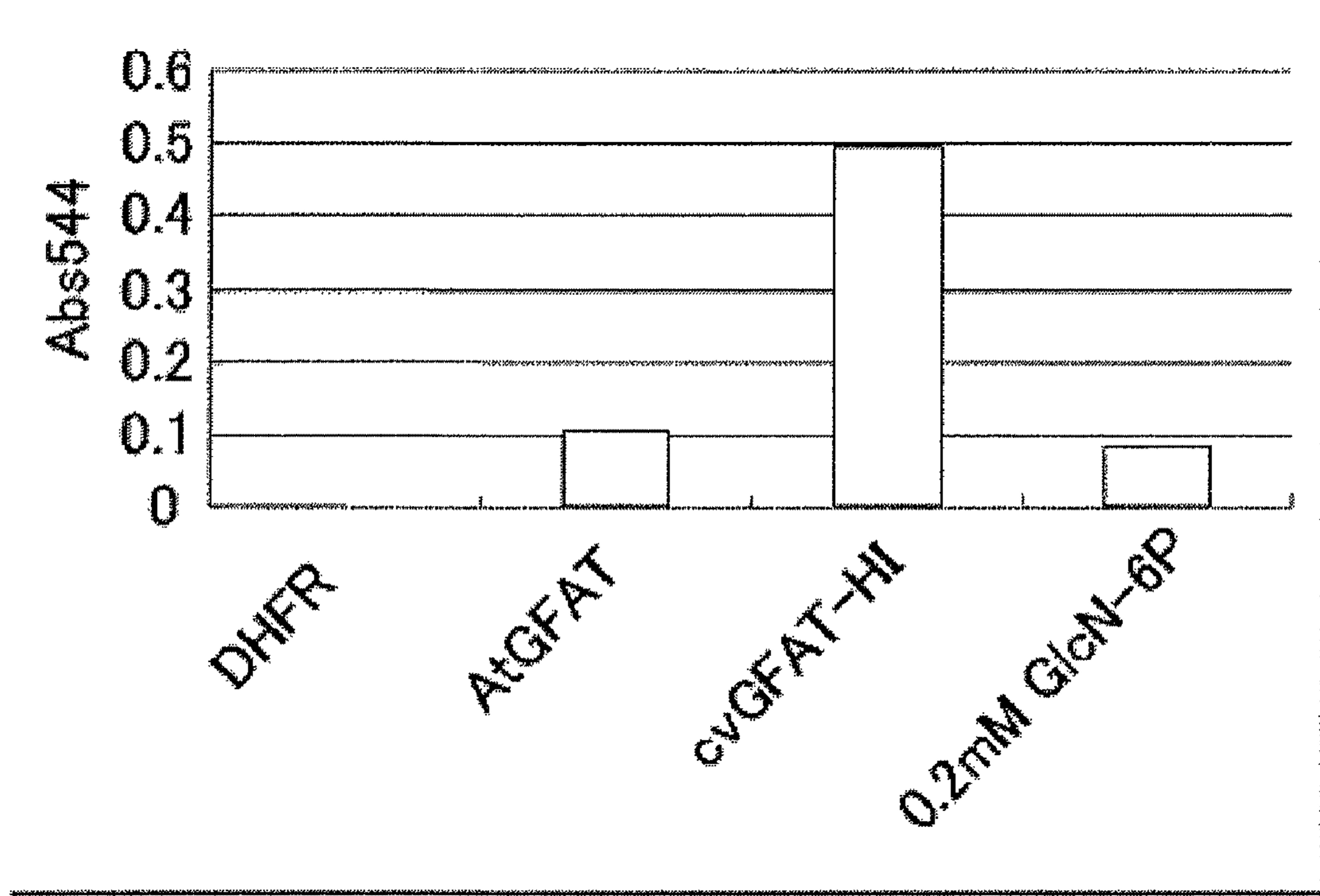


FIG. 5

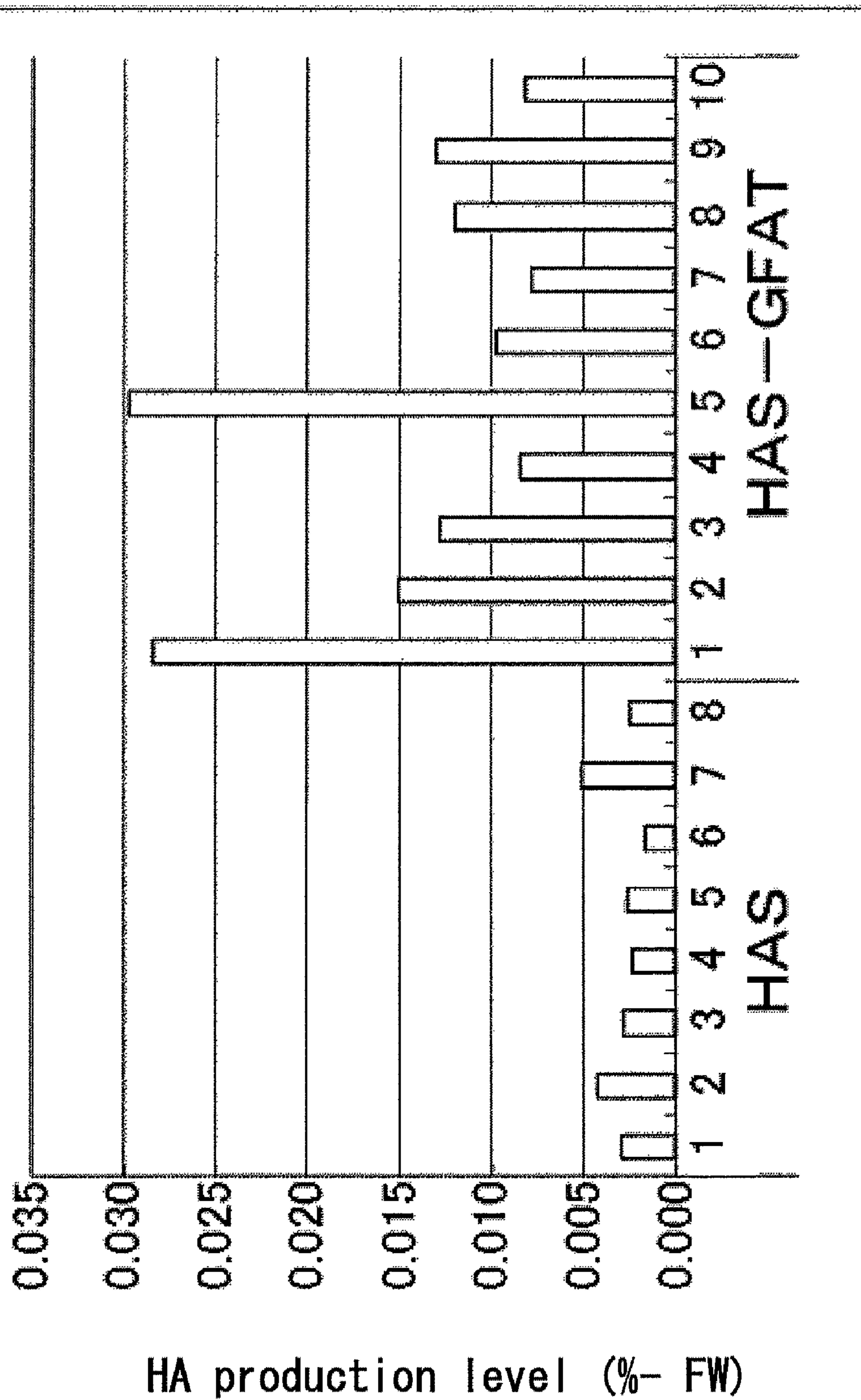
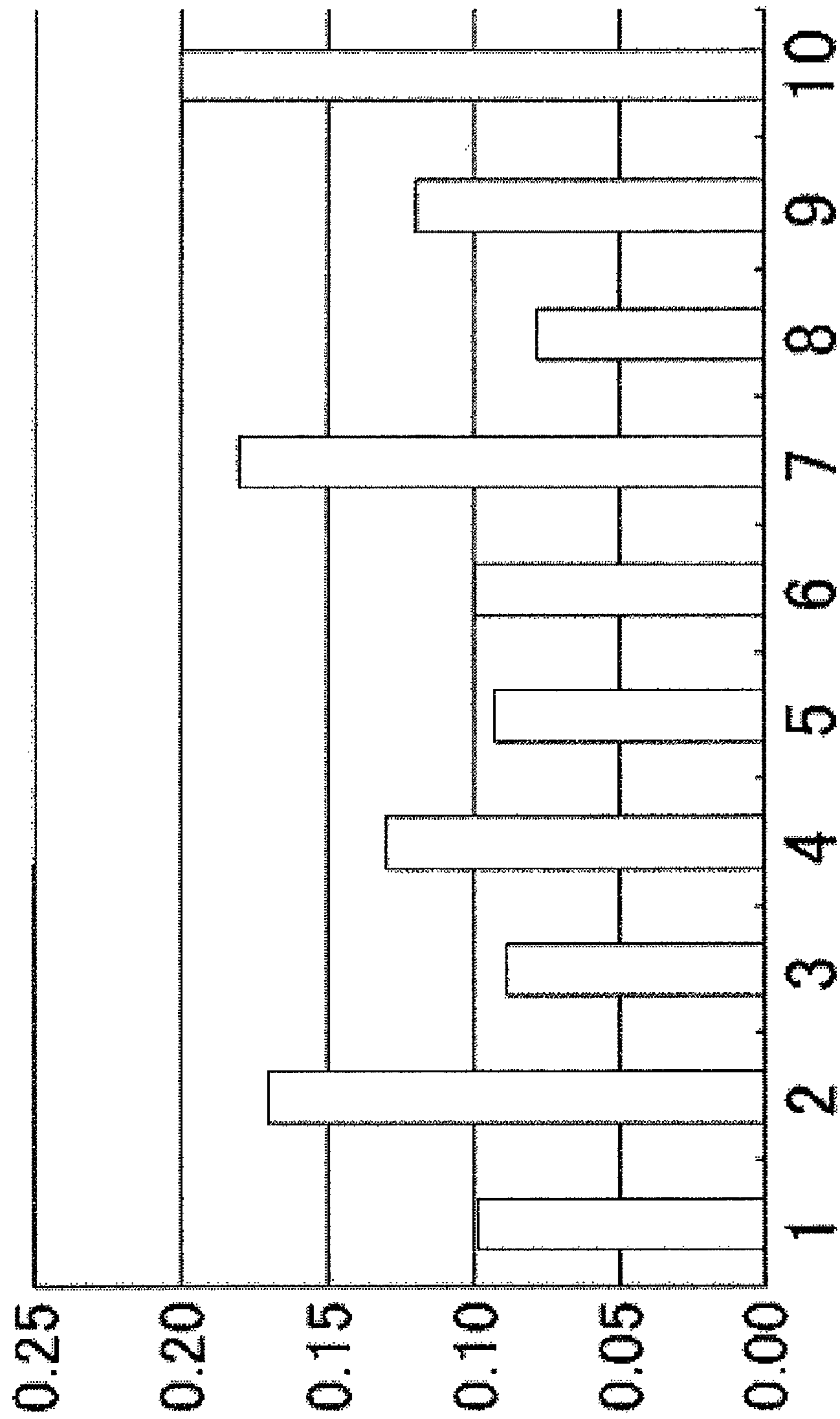


FIG. 6



HA production level (%- FW)

PLANT PRODUCING HYALURONIC ACID

TECHNICAL FIELD

The present invention generally relates to a method of producing hyaluronic acid in plants, transgenic plant cells or transgenic plants having an ability to produce hyaluronic acid, and methods of producing these transgenic cells and transgenic plants.

BACKGROUND ART

Hyaluronic acid is a glycosaminoglycan (mucopolysaccharide) isolated from the vitreous body of a bovine eye ball by Meyer and Palmer in 1934 (Meyer, K. and Palmer, J. W. (1934) J. Biol. Chem., 107, 629-634). High-molecular-weight hyaluronic acid has been used for the treatment of osteoarthritis, a surgery aid for ophthalmology, adhesion prevention, acceleration of wound healing and the like. It has been also reported that low-molecular-weight hyaluronic acid has physiologically active effects. New uses for hyaluronic acid as a biomaterial or in a medical application are expected to be found.

Until now, hyaluronic acid has been produced by extraction from mammalian tissues or microbial fermentation. However, risk of contamination with, for example, transmissible spongiform encephalopathies (prions) or transmission of viruses to humans has been concerned in the extraction from the mammalian tissues. Mammalian cells are expensive to grow and maintain. They require expensive growth media and grow slowly. Meanwhile, microbial fermentations have problems such as the requirement for sugar-containing growth medium and expensive facilities. In *Escherichia coli*, there are problems in that proteins are not processed, inclusion body might be formed, proteins are degraded by proteases, and the like. (Petrides, D. et al., (1995) Biotechnol. Bioeng., 48, 529). When therapeutic substances are produced in microorganisms, the purification costs become extremely expensive in order to prevent endotoxin contamination.

On the contrary, plants are ideal systems for producing carbohydrate with low energy load, in which carbohydrates are photosynthetically produced from water and carbon dioxide. The invention disclosed in Patent Document 6 shows that hyaluronic acid can be produced by introducing a hyaluronic acid synthase gene into plants or plant cells.

Patent Document 1: Japanese Unexamined Patent Application No. 1993-125103

Patent Document 2: Japanese Unexamined Patent Publication No. 1983-056692

Patent Document 3: Japanese Unexamined Patent Application No. 1997-319579

Patent Document 4: Japanese Unexamined Patent Application No. 1994-319580

Patent Document 5: Japanese Unexamined Patent Application No. 1997-056394

Patent Document 6: WO 05/012529

Nonpatent Document 1: Meyer, K. and Palmer, J. W., J. Biol. Chem., 107: 629-634, 1934

Nonpatent Document 2: Petrides, D. et al., Biotechnol. Bioeng., 48: 529, 1995

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows SDS-PAGE analysis of AtUGD before and after purification.

FIG. 2 shows SDS-PAGE analysis of cvUGD before and after purification.

FIG. 3 shows GFAT expressed using PROTEIOS (registered trademark).

FIG. 4 shows measurement of GFAT activity using the Reissig method.

FIG. 5 shows the hyaluronic acid production level of transgenic tobacco plants in which cvHAS-cvGFAT gene and cvHAS gene are introduced respectively.

FIG. 6 shows the hyaluronic acid production level of transgenic tobacco plants into which multiple genes have been introduced.

DISCLOSURE OF THE INVENTION

Problems to be Solved by the Invention

The primary object of the present invention is to provide plants and plant cells that can produce hyaluronic acid more effectively by improving previously known methods of producing hyaluronic acid in plants, the methods of producing thereof, and the recombinant expression vectors therefor.

Means for Solving the Problems

As a result of extensive study to solve the above problems, the present inventor has found that hyaluronic acid is produced extensively in the plants by transforming plants and plant cells with genes that encode proteins having enzymatic activity of producing hyaluronic acid and genes that encode proteins having enzymatic activity of synthesizing sugar-nucleotides, and further extensively study has achieved the present invention.

That is, the present invention relates to the following items.

1. A method of producing hyaluronic acid, comprising co-expressing a protein with hyaluronic acid synthase activity and an exogenous protein with sugar-nucleotide synthase activity in a plant cell or a plant.

2. A method of producing hyaluronic acid, containing the steps of:

(1) transforming a plant cell or a plant using a recombinant expression vector, the recombinant expression vector having DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under control of a promoter(s) capable of functioning in plants;

(2) growing a transformant obtained by the transformation; and

(3) isolating hyaluronic acid produced by the transformant.

3. The method of producing hyaluronic acid according to Item 2, wherein the promoter is an organ-specific or a tissue-specific promoter.

4. The method of producing hyaluronic acid according to Item 2 or 3, wherein the DNA encoding a protein with hyaluronic acid synthase activity is DNA of (a) or (b) below:

(a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 1 or 3; or

(b) DNA hybridizing to the nucleotide sequence complementary to DNA consisting of the nucleotide sequence of (a) under stringent conditions, and the DNA encoding a protein with hyaluronic acid synthase activity.

5. The method of producing hyaluronic acid according to any of Items 1 to 3, wherein the protein with hyaluronic acid synthase activity is a protein of (a) or (b) below:

(a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 2 or 4; or

(b) a protein having the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and the protein having hyaluronic acid synthase activity.

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6. The method of producing hyaluronic acid according to any of Items 1 to 5, wherein the sugar nucleotide is uridin-5'-diphospho(UDP)-N-acetylglucosamine and/or UDP-glucuronic acid.

7. The method of producing hyaluronic acid according to any of Items 1 to 6, wherein the protein with sugar-nucleotide synthase activity is at least one protein selected from the group consisting of glutamine:fructose-6-phosphate amidotransferase, UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridylyltransferase, inositol oxygenase, glucuronokinase and glucuronate-1-phosphate uridylyl transferase.

8. The method of producing hyaluronic acid according to any of Items 1 to 6, wherein the protein with sugar-nucleotide synthase activity is at least one protein selected from the group consisting of UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridylyltransferase, inositol oxygenase, glucuronokinase and glucuronate-1-phosphate uridylyl transferase, and glutamine:fructose-6-phosphate amidotransferase.

9. The method of producing hyaluronic acid according to any of Items 1 to 6, wherein a protein with sugar-nucleotide synthase activity is glutamine:fructose-6-phosphate amidotransferase and/or UDP-glucose dehydrogenase.

10. The method of producing hyaluronic acid according to any of Items 2 to 9, wherein the DNA encoding a protein with sugar nucleotide synthase activity is DNA derived from chlorella virus and/or *Arabidopsis thaliana*.

11. The method of producing hyaluronic acid according to any of Items 2 to 10, wherein the DNA encoding a protein with sugar-nucleotide synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 5, 7 or 9; or
- (b) DNA hybridizing to the nucleotide sequence complementary to DNA consisting of the nucleotide sequence of (a) under stringent conditions, and said DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity.

12. The method of producing hyaluronic acid according to any of Items 1 to 10, wherein the protein with sugar-nucleotide synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 6, 8 or 10; or
- (b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and the protein having glutamine:fructose-6-phosphate amidotransferase activity.

13. The method of producing hyaluronic acid according to any of Items 2 to 10, wherein the DNA encoding a protein with sugar-nucleotide synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 11, 13, 17, 19, or 21; or
- (b) DNA hybridizing to the nucleotide sequence complementary to DNA consisting of the nucleotide sequence of (a) under stringent conditions, and the DNA encoding a protein with UDP-glucose dehydrogenase activity.

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14. The method of producing hyaluronic acid according to any of Items 1 to 10, wherein the protein with sugar-nucleotide synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20 or 22; or
- (b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and the protein having UDP-glucose dehydrogenase activity.

15. The method of producing hyaluronic acid according to any of Items 1 to 14, wherein the plant is selected from the group consisting of angiosperms, gymnosperms, pteridophytes and bryophytes.

16. The method of producing hyaluronic acid according to Item 3, wherein organs are selected from the group consisting of roots, stems, stem tubers, leave, floral organs, tuberous roots, seeds and shoot apices.

17. The method of producing hyaluronic acid according to Item 3, wherein one or more tissues are selected from the group consisting of epidermis, phloem, soft tissues, xylem and vascular bundles.

18. A transgenic plant cell or a transgenic plant having an ability to produce hyaluronic acid by co-expressing a protein with hyaluronic acid synthase activity and an exogenous protein with sugar-nucleotide synthase activity, or a progeny thereof, or an organ or a tissue thereof having the same nature as in the plant.

19. A transgenic plant cell or a transgenic plant having an ability of producing hyaluronic acid, being transformed with an recombinant expression vector containing DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under control of a promoter capable of functioning in plants; a progeny having the same nature thereof; or an organ or a tissue thereof.

20. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to Item 19, wherein the promoter is an organ-specific or a tissue-specific promoter.

21. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to Item 19 or 20, wherein the DNA encoding a protein with hyaluronic acid synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 1 or 3; or
- (b) DNA hybridizing to the nucleotide sequence complementary to DNA consisting of the nucleotide sequence of (a) under stringent conditions, and the DNA encoding a protein with hyaluronic acid synthase activity.

22. The transgenic plant cell or the transgenic plant according to any of Items 18 to 20, wherein the protein with hyaluronic acid synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 2 or 4; or
- (b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and the protein having hyaluronic acid synthase activity.

23. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 22, wherein the sugar nucleotide is UDP-N-acetylglucosamine and/or UDP-glucuronic acid.

24. The transgenic plant cell or the transgenic plant according to any of Items 18 to 23, wherein the protein with sugar-nucleotide synthase activity is at least one protein selected

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from the group consisting of, glutamine:fructose-6-phosphate amidotransferase, UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridyltransferase, inositol oxygenase, glucuronokinase and glucuronate-1-phosphate uridyl transferase.

25. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 23, wherein the protein with sugar-nucleotide synthase activity is at least one protein selected from the group consisting of UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridyltransferase, inositol oxygenase, glucuronokinase and glucuronate-1-phosphate uridyl transferase, and glutamine:fructose-6-phosphate amidotransferase.

26. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 23, wherein the protein with sugar-nucleotide synthase activity is glutamine:fructose-6-phosphate amidotransferase and/or UDP-glucose dehydrogenase.

27. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 19 to 26, wherein DNA encoding a protein with sugar nucleotide synthase activity is derived from chlorella virus and/or *Arabidopsis thaliana*.

28. The transgenic plant cell or the transgenic plant; the progeny having the same nature thereof; or the organ or the tissue thereof according to any of Items 19 to 27, wherein DNA encoding a protein with sugar-nucleotide synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 5, 7 or 9; or
- (b) DNA hybridizing to the nucleotide sequence complementary to DNA consisting of the nucleotide sequence of (a) under stringent conditions, and the DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity.

29. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 27, wherein the protein with sugar-nucleotide synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 6, 8 or 10; or
- (b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and the protein having glutamine:fructose-6-phosphate amidotransferase activity.

30. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 19 to 27, wherein the DNA encoding a protein with sugar-nucleotide synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 11, 13, 15, 17, 19, or 21; or

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- (b) DNA hybridizing to DNA consisting of the base sequence complementary to the base sequence of (a) under stringent conditions, and the DNA encoding a protein with DP-glucose dehydrogenase activity.

31. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 27, wherein the protein with sugar-nucleotide synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20 or 22; or
- (b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and said protein with UDP-glucose dehydrogenase activity.

32. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 31, wherein the plant is any plant selected from the group consisting of gymnosperms, gymnosperms, pteridophytes and bryophytes.

33. The transgenic plant cell or the transgenic plant; the progeny having the same nature thereof; or the organ or the tissue thereof according to any of Items 18 to 31, wherein the organ is one or more organs selected from the group consisting of roots, stems, stem tubers, leaves, floral organs, tuberous roots, seeds and shoot apices.

34. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 31, wherein the tissue is one or more tissues selected from the group consisting of epidermis, phloem, soft tissues, xylem and vascular bundles.

35. Plant extract obtained from the transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to any of Items 18 to 34.

36. The plant extract according to Item 35, wherein the plant extract contains hyaluronic acid.

37. A recombinant expression vector, comprising DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under control of a promoter capable of functioning in plants.

38. The recombinant expression vector according to Item 37, wherein the promoter is an organ-specific, or a tissue-specific promoter.

39. The recombinant expression vector according to Item 37 or 38, wherein the DNA encoding a protein with hyaluronic acid synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 1 or 3; or
- (b) DNA hybridizing to DNA consisting of the nucleotide sequence complementary to the nucleotide sequence of (a) under stringent conditions, and the DNA encoding a protein with hyaluronic acid activity.

40. The recombinant expression vector according to Item 37 or 38, wherein the protein with hyaluronic acid synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 2 or 4; or
- (b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and the protein having hyaluronic acid synthase activity.

41. The recombinant expression vector according to any of Items 37 to 40, wherein the sugar nucleotide is UDP-N-acetylglucosamine and/or UDP-glucuronic acid.

42. The recombinant expression vector according to any of Items 37 to 41, wherein the protein with sugar-nucleotide synthase activity is at least one protein selected from the group consisting of glutamine:fructose-6-phosphate amidotransferase, UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridylyltransferase, inositol oxygenase, glucuronokinase and glucuronate-1-phosphate uridylyl transferase.

43. The recombinant expression vector according to any of Items 37 to 41, wherein the protein with sugar-nucleotide synthase activity is at least one protein selected from the group consisting of UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridylyltransferase, inositol oxygenase, glucuronokinase and glucuronate-1-phosphate uridylyl transferase, and glutamine:fructose-6-phosphate amidotransferase.

44. The recombinant expression vector according to any of Items 37 to 41, wherein the protein with sugar-nucleotide synthase activity is glutamine:fructose-6-phosphate amidotransferase and/or UDP-glucose dehydrogenase.

45. The recombinant expression vector according to any of Items 37 to 41, wherein the DNA encoding a protein with sugar nucleotide synthase activity is DNA derived from chloro-*re*lla virus and/or *Arabidopsis thaliana*.

46. The recombinant expression vector according to any of Items 37 to 44, wherein the DNA encoding a protein with sugar nucleotide synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 5, 7 or 9; or
- (b) DNA hybridizing to DNA consisting of the nucleotide sequence complementary to the nucleotide sequence of (a) under stringent conditions, and said DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity.

47. The recombinant expression vector according to any of Items 37 to 44, wherein the protein with sugar-nucleotide synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting an amino acid sequence represented by SEQ ID NO: 6, 8 or 10; or
- (b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and the protein having glutamine:fructose-6-phosphate amidotransferase activity.

48. The recombinant expression vector according to any of Items 37 to 44, wherein the DNA encoding a protein with sugar nucleotide synthase activity is DNA of (a) or (b) below:

- (a) DNA consisting of a nucleotide sequence represented by SEQ ID NO: 11, 13, 15, 17, 19, or 21; or
- (b) DNA hybridizing to DNA consisting of the nucleotide sequence complementary to the nucleotide sequence of (a) under stringent conditions, and said DNA encoding a protein with UDP-glucose dehydrogenase activity.

49. The recombinant expression vector according to any of Items 37 to 44, wherein the protein with sugar-nucleotide synthase activity is a protein of (a) or (b) below:

- (a) a protein consisting of an amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20 or 22; or

(b) a protein consisting of the amino acid sequence of (a) with one or a few amino acids deleted, substituted or added, and said protein having UDP-glucose dehydrogenase activity.

50. A method of producing transgenic plant cell or the transgenic plant having an ability to produce hyaluronic acid, the method comprising transforming a plant cell or a plant using any vector according to Items 37 to 49.

51. A cosmetic composition containing hyaluronic acid as an active agent, wherein the hyaluronic acid is obtained by any of the methods of producing hyaluronic acid according to Items 1 to 17.

Effects of the Invention

According to the present invention, hyaluronic acid, which is not naturally produced in plants, is produced in plants. According to the present invention, a gene encoding a protein with hyaluronic acid synthase activity and a gene encoding a protein with sugar-nucleotide synthase activity are expressed in plants, so that hyaluronic acid is highly produced in plants. Plants or cultured plant cells capable of producing more hyaluronic acid than by conventional methods, a method thereof, and a recombinant expression vector thereof can be provided. Therefore, the present invention can provide plant-produced safe hyaluronic acid at low cost.

BEST MODE FOR CARRYING OUT THE INVENTION

A feature of the present invention is a method of producing hyaluronic acid, containing co-expressing a protein with hyaluronic acid synthase activity and an exogenous protein with sugar nucleotide synthase activity in plant cells or plants so as to obtain hyaluronic acid.

Another feature of the present invention is a method of producing hyaluronic acid containing:

- (1) transforming plant cells or plants using a recombinant expression vector that contains DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants;
- (2) growing the transformant obtained by the transformation; and
- (3) isolating hyaluronic acid produced in the transformants.

Yet another feature of the present invention is cosmetic compositions that contain hyaluronic acid as an active agent, wherein the hyaluronic acid is obtained by the above method for producing hyaluronic acid.

Yet another feature of the present invention is a recombinant expression vector for producing hyaluronic acid, wherein the recombinant expression vector contains DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants.

Some features of the present invention are transgenic plant cells or transgenic plants, the progenies having the same nature thereof, or the organs or the tissues thereof, wherein the transformants have obtained an ability to produce hyaluronic acid by co-expressing a protein with hyaluronic acid synthase activity and an exogenous protein with sugar-nucleotide synthase activity.

A feature of the present invention is transgenic plant cells or transgenic plants, the progenies having the same nature

thereof, or the organs or the tissues thereof, wherein the transformants are transformed using a recombinant expression vector containing DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants.

Another feature of the present invention is a method for producing transgenic plant cells or transgenic plants that have an ability to produce hyaluronic acid. The method includes transforming plant cells or plants using a recombinant expression vector that contains DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants.

The following explains the present invention in detail.
Hyaluronic Acid Synthase

In the present invention, plant cells or plants are transformed using DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants.

In the present invention, a protein with hyaluronic acid synthase activity synthesizes hyaluronic acid using UDP-glucuronic acid and UDP-N-acetylglucosamine as substrates. The hyaluronic acid has a polymer structure consisting of repeated units of glucuronic acid and N-acetylglucosamine.

In the present invention, a protein with hyaluronic acid synthase activity is, as long as the protein has the above mentioned nature, not particularly limited. Hyaluronic acid synthase (hereinafter be occasionally abbreviated as HAS) derived from animals, microorganisms, viruses and the like can be used. Particularly, hyaluronic acid synthase derived from vertebrates such as humans, mice, rabbits, chickens, cattle and *Xenopus laevis*, microorganisms such as *Streptococcus* and *Pasteurella*, viruses such as chlorella virus and the like can be used.

More specifically, examples of the protein with hyaluronic acid synthase activity are HAS (A98R) derived from chlorella virus PBCV-1; HAS1, HAS2 and HAS3 of the hyaluronic acid synthase (hHAS) derived from humans; HAS1, HAS2 and HAS3 of the mouse derived hyaluronic acid synthase (mHAS); HAS1, HAS2 and HAS3 of the chicken derived hyaluronic acid synthase (gHAS); HAS2 of the rat derived hyaluronic acid synthase (rHAS); HAS2 of the cattle derived hyaluronic acid synthase (bHAS); HAS1, HAS2 and HAS3 of the *Xenopus laevis* derived hyaluronic acid synthase (xHAS); the *Pasteurella multocida* derived hyaluronic acid synthase (pmHAS); the *Streptococcus pyogenes* derived hyaluronic acid synthase (spHAS); and the hyaluronic acid synthase (seHAS) gene derived from *Streptococcus equisimilis*. There are various types of hyaluronic acid synthase (HAS) genes such as HAS1, HAS2 and HAS3, however, the type is not particularly limited. Any of the above described HAS can be used, among which the chlorella virus derived HAS is preferable, chlorella virus derived HAS which are shown by a protein consisting of an amino acid sequence represented by SEQ ID NO: 2 or 4 is more preferable.

The protein consisting of an amino acid sequence represented by SEQ ID NO: 2 or 4 may be a protein having one or a few amino acids deleted, substituted or added, as long as hyaluronic acid synthase activity is not lost. For example, the amino acid sequence represented by SEQ ID NO: 2 or 4 may have a deletion of at least one amino acid, preferably 1 to 10 amino acids, more preferably 1 to 5 amino acids, an addition of at least one amino acid, preferably 1 to 10 amino acids, more preferably 1 to 5 amino acids, or a substitution of at least one amino acid, preferably 1 to 10 amino acids, more prefer-

ably 1 to 5 amino acids by other amino acids. However, mutations are not limited to the above. Such mutations include artificial mutations other than naturally occurring mutations. For example, it is reported that hyaluronic acid synthase derived from *Pasteurella multocida* has hyaluronic acid synthase activity even if about 270 amino acids in the putative membrane-bound domain and the putative transmembrane domain are deleted (Jing et al., 2000, *Glycobiology*, 10, 883-889). The number of mutated amino acids is not limited, as long as the hyaluronic acid synthase activity is not lost. HAS may be a protein consisting of a part of the amino acid sequence represented by SEQ ID NO: 2 or 4, having hyaluronic acid synthase activity.

Hyaluronic acid synthase activities are determined as follows. For the reaction, samples are incubated in 0.2 ml of 50 mM Tris-HCl buffer (pH7.0) containing 1 mM dithiothreitol, 20 mM magnesium chloride, 1 mM ethylene glycol bis (β -aminoethyl ether)-N,N,N,N-tetra-acetic acid, 15% glycerol, 0.5 mM uridine-5'-diphosphoglucuronic acid (hereinafter abbreviated as UDP-GlcA), 0.5 mM uridine-5'-diphospho-N-acetylglucosamine (hereinafter abbreviated as UDP-GlcNAc below), 0.1 μ M UDP-[¹⁴C]GlcA, 0.24 μ M UDP-[³H]GlcNAc, and 125 μ g of glucuronic acid for 1 hour. After the incubation, the reaction is terminated by boiling for 10 minutes. The reaction mixture is divided into two, 0.5 units of hyaluronidase (Seikagaku Corporation) derived from *Streptococcus dysgalactiae* is added to one of the solutions, and then incubated at 30° C. for 4 hours. Then the reaction solution is boiled for 10 minutes to inactivate the hyaluronidase. The reaction mixture is fractionated per 0.5 ml using Superdex Peptide HR10/30 (produced by Amersham Pharmacia Biotech Inc.) column chromatography (elute: 0.2M ammonium acetate). Each fraction is measured for radioactivity. As a result, hyaluronic acid activity can be determined from the sample reaction mixture based on the amounts of low-molecular-weight products digested by the hyaluronidase. Hyaluronic acid synthase activity can be also determined using the sandwich method, in which hyaluronic acid produced is measured using hyaluronic acid binding proteins.

According to the present invention, the DNA encoding a protein with hyaluronic acid synthase and is DNA encoding a protein that synthesizes hyaluronic acid from UDP-glucuronic acid and UDP-N-acetylglucosamine as substrates, wherein the hyaluronic acid has a polymer structure consisting of repeated units of glucuronic acid and N-acetylglucosamine.

According to the present invention, the DNA encoding a protein with hyaluronic acid synthase activity is, as long as the protein has the above mentioned properties, not particularly limited. Hyaluronic acid synthase (hereinafter occasionally abbreviated as HAS) genes derived from animals, microorganisms, virus and the like can be used. For example, the hyaluronic acid synthase gene derived from vertebrates such as humans, mice, rabbits, chickens, cattle and *Xenopus laevis*, microorganisms such as *Streptococcus* and *Pasteurella*, and viruses such as chlorella virus and the like, can be used.

More specifically, HAS (A98R) genes derived from chlorella virus strain PBCV-1; HAS1, HAS2 and HAS3 of the hyaluronic acid synthase (hHAS) gene derived from humans; HAS1, HAS2 and HAS3 of the mouse derived hyaluronic acid synthase (mHAS) gene; HAS1, HAS2 and HAS3 of the chicken derived hyaluronic acid synthase (gHAS) gene; HAS2 of the rat derived hyaluronic acid synthase (rHAS) gene; HAS2 of the cattle derived hyaluronic acid synthase (bHAS) gene; HAS1, HAS2 and HAS3 of the *Xenopus laevis* derived hyaluronic acid synthase (xHAS) gene; the *Pasteurella multocida* derived hyaluronic acid synthase (pm-

HAS) gene; the *Streptococcus pyogenes* derived hyaluronic acid synthase (spHAS) gene; the hyaluronic acid synthase (seHAS) gene derived from *Streptococcus equisimilis* and the like are included. There are various types of hyaluronic acid synthase (HAS) genes such as HAS1, HAS2 and HAS3, however, the type is not particularly limited.

Any of the above described HAS can be used, among which the chlorella virus derived HAS gene is preferable, chlorella virus derived HAS genes which are shown by DNA consisting of a nucleotide sequence represented by SEQ ID NO: 1 or 3 are more preferable.

The DNA may be DNA that hybridizes with DNA consisting of the nucleotide sequence complementary to the nucleotide sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and encodes a protein with hyaluronic acid synthase activity.

The term "stringent conditions" above means conditions in which only a nucleotide sequence that encodes a polypeptide having hyaluronic acid synthase activity that is equivalent to that of hyaluronic acid synthase having a nucleotide sequence represented by SEQ ID NO: 1 or 3 forms a hybrid with the particular sequence (that is a specific hybrid), whereas a nucleotide sequence that encodes a polypeptide having non-equivalent activity does not form a hybrid with the particular sequence (that is a non-specific hybrid). Persons skilled in the art can easily choose such conditions by changing the temperatures of the hybridization reaction and washing, and the salt concentrations of hybridization reaction solutions and washing solutions, and the like. Specifically, one example of the stringent conditions of the present invention is the conditions in which hybridizing is performed in 6×SSC (0.9M NaCl, 0.09M trisodium citrate) or 6×SSPE (3M NaCl, 0.2M NAH_2PO_4 , 20 mM EDTA.2Na, pH7.4) at 42° C., and further washing is performed using 0.5×SSC at 42° C., however, the stringent conditions are not limited to such conditions.

The stringent conditions are preferably highly stringent conditions. The highly stringent conditions are not particularly limited, as long as a gene encoding A98R does not hybridize. For example, the highly stringent conditions are conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C. Sugar Nucleotide

Examples of usable sugar nucleotides are UDP-N-acetylglucosamine, UDP-glucuronic acid, UDP-N-acetylgalactosamine, UDP-glucose, UDP-galactose, UDP-xylose, GDP-fucose, GDP-mannose, CMP-neuraminic acid, and the like, however, the sugar nucleotide is not limited to these. Among these sugar nucleotides, UDP sugar is preferable, UDP-N-acetylglucosamine and UDP-glucuronic acid are more preferable.

By improving the production levels of such sugar nucleotides, the amounts of UDP-glucuronic acid and UDP-N-acetylglucosamine that are substrates for hyaluronic acid synthase are increased in plant cells or plants. To improve the production levels of such sugar nucleotide, a sugar nucleotide biosynthetic pathway enzyme, that is, a protein with sugar-nucleotide synthase activity is introduced into plant cells or plants.

Furthermore, in the present invention, it is found that simultaneous introduction of a protein with hyaluronic acid synthase activity into plant cells or plants enables increased hyaluronic acid synthesis using the increased UDP-glucuronic acid and UDP-N-acetylglucosamine as substrates, wherein the hyaluronic acid has a polymer structure consisting of repeated units of glucuronic acid and N-acetylglucosamine.

Enzymes Associated with Sugar Nucleotide Biosynthesis Pathway

According to the present invention, plant cells or plants are transformed with DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants.

Proteins with sugar-nucleotide synthase activity such as UDP-N-acetylglucosamine, UDP-glucuronic acid, UDP-N-acetylgalactosamine, UDP-glucose, UDP-galactose, UDP-xylose, GDP-fucose, GDP-mannose and CMP-neuraminic acid may be used. Proteins having synthase activity directed to, among these sugar nucleotides, UDP-N-acetylglucosamine, UDP-glucuronic acid, UDP-N-acetylgalactosamine, UDP-glucose, UDP-galactose, UDP-xylose are preferable. Proteins having sugar-nucleotide synthase activity directed to sugars, UDP-N-acetylglucosamine and UDP-glucuronic acid are more preferable.

The protein having sugar-nucleotide synthase activity of the present invention may be an enzyme catalyzing the reactions that are associated with sugar nucleotide biosynthesis pathways. Examples of the enzymes are glutamine:fructose-6-phosphate amidotransferase, UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate-N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridyltransferase, inositol oxygenase, glucuronokinase, glucuronate-1-phosphate uridyl transferase and the like. At least one protein selected from the group consisting of such proteins with sugar-nucleotide synthase activity may be expressed in plant cells or plants.

As a protein with sugar-nucleotide synthase activity, at least glutamine:fructose-6-phosphate amidotransferase may also be selected, and expressed in combination with a protein having other sugar-nucleotide synthase activity in plant cells or plants. Examples of other proteins having sugar-nucleotide synthase activity than glutamine:fructose-6-phosphate amidotransferase are at least one amino-acid protein selected from the group consisting of UDP-N-acetylglucosamine diphosphorylase, phosphoacetyl glucosamine mutase, glucosamine-6-phosphate N-acetyltransferase, glucosamine-1-phosphate N-acetyltransferase, phosphoglucomutase, N-acetylglucosamine kinase, hexokinase, N-acetylglucosamine acetylase, UDP-glucose dehydrogenase, UDP-glucose-1-phosphate uridyltransferase, inositol oxygenase, glucuronokinase, glucuronic acid-1-phosphate uridyl transferase and the like.

To achieve the intended effects of the present invention, at least, glutamine:fructose-6-phosphate amidotransferase is selected as a protein having the sugar-nucleotide synthase activity, thereby a better effect than that of conventional methods is obtained. More preferably, both of glutamine:fructose-6-phosphate amidotransferase (hereinafter occasionally abbreviated as GFAT) and UDP-glucose dehydrogenase (hereinafter occasionally abbreviated as UGD) are selected. The single use of UDP-glucose dehydrogenase also shows more enhanced effects than that of conventional methods.

Glutamine:Fructose-6-Phosphate Amidotransferase

The protein having glutamine:fructose-6-phosphate amidotransferase activity of the present invention is a protein that synthesizes glucosamine-6-phosphate from L-glutamine and fructose-6-phosphate as substrates.

The protein having glutamine:fructose-6-phosphate amidotransferase activity of the present invention is, as long as the protein has the above mentioned nature, not particularly

limited. Examples of such proteins are GFAT derived from eukaryotes, prokaryotes, viruses and the like. GFAT derived from eukaryotes such as humans, mice, corns, *Arabidopsis thaliana*, *Caenorhabditis elegans* and *Saccharomyces cerevisiae*, GFAT derived from prokaryotes such as *Bacillus subtilis* and *Escherichia coli*, and GFAT derived from viruses such as chlorella virus can be used, however, the protein is not limited to these.

The GFAT above can be preferably used, among which the GFAT derived from chlorella virus or *Arabidopsis thaliana* is more preferable. The chlorella virus derived GFAT shown by a protein consisting of the amino acid sequence represented by SEQ ID NO: 6 or 8, and the *Arabidopsis thaliana* derived GFAT shown by a protein consisting of an amino acid sequence represented by SEQ ID NO: 10 are especially preferable.

The protein may also be a protein consisting of the amino acid sequence represented by SEQ ID NO: 6, 8 or 10, or the protein with one or a few amino acids deleted, substituted or added to its amino acid sequence as long as the glutamine:fructose-6-phosphate amidotransferase activity is not lost. For example, the amino acid sequence represented by SEQ ID NO: 6, 8 or 10 may have a deletion of at least one amino acid, preferably 1 to 10 amino acids, and more preferably 1 to 5 amino acids. The amino acid sequence represented by SEQ ID NO: 6, 8 or 10 may have an addition of at least one amino acid, preferably 1 to 10 amino acids, more preferably 1 to 5 amino acids. The amino acid sequence represented by SEQ ID NO: 6, 8 or 10 may have a substitution of at least one amino acid, preferably 1 to 10 amino acids, more preferably 1 to 5 amino acids, and more preferably 1 to 5 amino acids, with other amino acids. However, mutations are not limited to the aforementioned examples. Such mutations include artificial mutations other than naturally occurring mutations. The number of mutated amino acids is, as long as the GFAT activity is not lost, not particularly limited. An example of naturally occurring mutations is the GFAT derived from chlorella virus strain Hirosaki and having a sequence of SEQ ID NO: 5 that departs from known GFAT of chlorella virus strain PBCV-1 and K21 at least by 2% in their amino acid sequences due to mutation. The protein may be a protein having a part of the amino acid sequence represented by SEQ ID NO: 6, 8 or 10, and having glutamine:fructose-6-phosphate amidotransferase activity.

The Glutamine:fructose-6-phosphate amidotransferase activity can be evaluated by adding the enzyme solution to reaction mixture (pH7.0) containing fructose-6-phosphate (15 mM), L-glutamine (15 mM), EDTA (1 mM), DTT (1 mM) and KH_2PO_4 (60 mM), and then incubating at 37° C. for a few hours, and subsequently measuring the amount of glucosamine-6-phosphate or glutamic acid produced. Specifically, examples of the methods are the Reissig method (J. Biol. Chem, 1955, 217(2), 959-66), which is a modified version of the Morgan & Elson method for measuring glucosamine-6-phosphate; enzymatic analysis (J Biochem Biophys Methods, 2004, 59(3), 201-8) for measuring glutamic acid using glutamic acid dehydrogenase; and the like.

DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity of the present invention is DNA encoding a protein that has enzyme activity to synthesize glucosamine-6-phosphate from L-glutamine and fructose-6-phosphate as substrates.

DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity of the present invention is, as long as the proteins have the above mentioned properties, not particularly limited. Examples of such GFAT genes are GFAT genes derived from eukaryotes, prokaryotes, viruses and the

like. GFAT genes derived from eukaryotes such as humans, mice, corns, *Arabidopsis thaliana*, *Caenorhabditis elegans* and *Saccharomyces cerevisiae*, GFAT genes derived from prokaryotes such as *Bacillus subtilis* and *Escherichia coli*, GFAT genes derived from viruses such as chlorella virus and the like can be used. There are various types of GFAT genes such as GFAT1 and GFAT2, however, the type is not particularly limited.

The above described GFAT genes can be preferably used, among which the GFAT gene derived from Chlorella virus or derived from *Arabidopsis thaliana* is more preferable. The GFAT gene derived from Chlorella virus and consisting of the nucleotide sequence represented by SEQ ID NO: 5 or 7, or the GFAT gene derived from *Arabidopsis thaliana* and consisting of the nucleotide sequence represented by SEQ ID NO: 9 is especially preferable.

The DNA may also be DNA hybridizing with a nucleotide sequence complementary to the nucleotide sequence represented by SEQ ID NO: 5, 7 or 9 under stringent conditions, and encoding a protein with GFAT activity.

The term "stringent conditions" above means conditions in which only a nucleotide sequence encoding a polypeptide with a glutamine:fructose-6-phosphate amidotransferase activity equivalent to that of glutamine:fructose-6-phosphate amidotransferase represented by SEQ ID NO: 5, 7 or 9 forms a hybrid with the particular sequence (i.e., a specific hybrid), whereas a nucleotide sequence encoding a polypeptide with non-equivalent activity does form a hybrid with the particular sequence (i.e., a non-specific hybrid). Persons skilled in the art can easily choose such conditions by changing temperatures for the hybridization reaction and washing, salt concentrations of hybridization reaction solutions and washing solutions, and the like. One example of the stringent conditions of the present invention is conditions in which hybridization is performed using 6×SSC (0.9M NaCl, 0.09M trisodium citrate) or 6×SSPE (3M NaCl, 0.2M NaH_2PO_4 , 20 mM EDTA.2Na, pH7.4) at 42° C., and subsequently washing is performed using 0.5×SSC at 42° C., however, the stringent conditions are not limited to these.

The stringent conditions are preferably highly stringent conditions. The "highly stringent conditions" are for example, conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC and 0.1% SDS at 60° C.

UDP-Glucose Dehydrogenase

The protein having UDP-glucose dehydrogenase activity of the present invention may be a protein that synthesizes UDP-glucuronic acid from UDP-glucose as a substrate.

The protein having UDP-glucose dehydrogenase activity of the present invention is, as long as the protein has the above-mentioned nature, not particularly limited. Examples of the proteins are UGD derived from eukaryotes, prokaryotes, viruses and the like. UGD derived from eukaryotes such as UGD derived from humans, cattle mice, poplars, sugarcanes and *Arabidopsis thaliana* UGD derived from prokaryotes such as *Escherichia coli*, *Pasteurella multocida* and *Lactobacillus lactis*, and UGD derived from viruses such as chlorella virus can be used, however, the protein of the invention is not limited to these.

The UGD described above can be preferably used, among which UGD derived from chlorella virus or *Arabidopsis thaliana* is more preferable. UGD derived from chlorella virus and shown by a protein consisting of an amino acid sequence represented by SEQ ID NO: 12 or 14, or UGD derived from *Arabidopsis thaliana* shown by a protein consisting of an amino acid sequence represented by SEQ ID NO: 16, 18, 20 or 22 is particularly preferable.

The protein may be a protein consisting of the amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20, or 22, or a protein having a mutation such as a deletion of one or a few amino acids, a substitution, or an addition as long as the UDP-glucose dehydrogenase activity is not lost. For example, the amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20, or 22 may have a deletion of at least one amino acid, preferably 1 to 10 amino acids, and more preferably 1 to 5 amino acids. The amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20, or 22 may have an addition of at least one amino acid, preferably 1 to 10 amino acids, and more preferably 1 to 5 amino acids. The amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20, or 22 may also have a substitution of at least 1 amino acid, preferably 1 to 10 amino acids, and more preferably 1 to 5 amino acids by other amino acids. However, the mutations are not limited to the above. Such mutations also include artificial mutations other than naturally occurring mutations. The number of mutated amino acids is not particularly limited, as long as the UGD activity is not lost. The protein may also be a protein having a part of the amino acid sequence represented by SEQ ID NO: 12, 14, 16, 18, 20, or 22, and having UDP-glucose dehydrogenase.

UDP-glucose dehydrogenase activity is evaluated by adding the enzyme solution to reaction mixture (pH 8.0) containing UDP-glucose (4 mM), NAD⁺ (1 mM), EDTA (1 mM) and Tris-HCl (20 mM), performing the reaction at 37° C., and subsequently measuring the UDP-glucuronic acid or NADH produced. Specifically, NADH can be measured according to Tenhaken and Thulke's report (Plant Physiol. 1996, 112: 1127-34).

The DNA encoding a protein with UDP-glucose dehydrogenase activity of the present invention is DNA encoding a protein that has enzyme activity to synthesize UDP-glucuronic acid from UDP-glucose as a substrate.

The DNA encoding a protein with UDP-glucose dehydrogenase activity of the present invention is, as long as the protein has the above-mentioned nature, not particularly limited. Examples of the DNA are UGD genes derived from eukaryotes, prokaryotes, viruses and the like. UGD genes derived from eukaryotes such as humans, cattle, mice, poplars, sugarcane and *Arabidopsis thaliana*, UGD genes derived from prokaryotes such as *Escherichia coli*, *Pasteurella multocida* and *Lactobacillus lactis*, and UGD genes derived from viruses such as chlorella virus and the like can be used, however, the UGD genes are not limited to these.

The UGD genes described above can be preferably used, among which UGD gene derived from chlorella virus or *Arabidopsis thaliana* is more preferable, UGD gene derived from chlorella viruses and having a nucleotide sequence represented by SEQ ID NO: 11, or 13 or UGD gene derived from *Arabidopsis thaliana* having a nucleotide sequence represented by SEQ ID NO: 15, 17, 19 or 21 is particularly preferable.

The DNA may be DNA hybridizing with DNA consisting of a nucleotide sequence complementary to the nucleotide sequences represented by SEQ ID NO: 11, 13, 15, 17, 19, or 21 under stringent conditions, and encoding a protein with UDP-glucose dehydrogenase activity.

The "stringent conditions" described above are conditions in which only a nucleotide sequence encoding a polypeptide with the activity equivalent to that of UDP-glucose dehydrogenase represented by SEQ ID NO: 11, 13, 15, 17, 19, or 21 forms a particular hybrid with the particular sequence (i.e., a specific hybrid), whereas a nucleotide sequence encoding a polypeptide with non-equivalent activity does not form a hybrid with the particular sequence (i.e., a non-specific

hybrid). Persons skilled in the art can easily choose such conditions by changing the temperatures for hybridization reaction and washing, adjusting the salt concentrations of the hybridization reaction solutions and washing solutions, and the like. One example of stringent conditions is conditions in which hybridization is performed using 6×SSC (0.9M NaCl, 0.09M trisodium citrate) or 6×SSPE (3M NaCl, 0.2M NAH₂PO₄, 20 mM EDTA).2Na, pH7.4) at 42° C., and then washing is performed using 0.5×SSC. However, the stringent conditions are not limited to these.

The stringent conditions are preferably highly stringent conditions. Highly stringent conditions are, for example, conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

A Recombinant Expression Vector and Transformation

Transgenic plant cells or transgenic plants capable of producing hyaluronic acid, progenies, or organs or tissues thereof having the same nature thereof can be obtained by transforming hosts using a recombinant expression vector. The recombinant expression vector contains DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants.

A protein having hyaluronic acid synthase activity and a protein with sugar-nucleotide synthase activity are expressed in the above transgenic plant cells or the transgenic plants, the progenies, or the organs or the tissues thereof having the same nature thereof.

"An exogenous protein with sugar-nucleotide synthase activity" is, unlike an endogenous protein, a foreign protein having sugar nucleotide synthase activity and being newly introduced into plant cells or plants from outside. An example of a method for "newly introducing such a gene from outside" includes transformation using a recombinant expression vector and the like. "Newly introducing a gene from outside" includes transforming an endogenous gene from outside of the cell using a recombinant expression vector. Since there have been no proteins with hyaluronic acid synthase activity found in plant cells and plants, it is obvious that the protein is exogenous without the description.

The above transgenic plant cells or transgenic plants, the progenies, or the organs or tissues thereof having the same nature thereof enable high-level production of hyaluronic acid. This is demonstrated below.

In the present invention, the hosts mean any whole plants, seeds, plant organs (for example, petals, roots, stems, stem tubers, leaves, floral organs, tuberous roots, seeds, shoot apices and the like), plant tissues (for example, epidermis, phloem, soft tissues, xylem, vascular bundles and the like), or cultured plant cells.

In the present specification, plants mean any multicellular plants including spermatophytes, gymnosperms, pteridophytes, bryophytes, lichens and the like, and include any whole plants, seeds, plant organs (for example, petals, roots, stems, stem tubers, leaves, floral organs, tuberous roots, seeds, shoot apices and the like), plant tissues (for example, epidermis, phloem, soft tissues, xylem, vascular bundles and the like), or cultured plant cells.

Hyaluronic acid may also be produced thus by culturing the transformants resulting from the transformation, and isolating the produced hyaluronic acid thereby.

Vectors that are generally used for producing transgenic plant cells or transgenic plants can be used as recombinant expression vectors.

Such vectors are not particularly limited as long as the vectors comprise a promoter sequence capable of being tran-

scribed in plant cells and a polyadenylation site required for stabilizing transcripts. For example, vectors such as “pBI121”, “pBI221”, “pBI101” and “pIG121Hm” can be used.

When cultured plant cells are used as hosts, transformation can be achieved by introducing a recombinant expression vector for producing hyaluronic acid in cultured plant cells by the electroporation method, the *Agrobacterium* binary vector method or the Particle Bombardment method. The vector includes DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity placed under the control of a promoter(s) capable of functioning in plants. The plant cells in which the expression vector has been introduced are, for example, selected on the basis of resistance to antibiotics such as kanamycin and the like. The transformed plant cells can be used for cell culture, tissue culture and organ culture. It is also possible to regenerate plants using a previously known plant tissue culture method.

Examples of plant cells subjected to the transformation include BY-2 cells and T-13 cells derived from tobacco, kurodagosun cells derived from carrots, VR cells and VW cells derived from grapes, PAR cells, PAP cells and PAW cells derived from *Phytolacca americana* L., T87 cells derived from *Arabidopsis thaliana*, Asp-86 cells, A. per cells, A. pas cells and A. plo cells derived from asparagus, Cba-1 cells derived from watermelon, Sly-1 cells derived from tomatoes, 1-Mar cells derived from peppermint, CRA cells and V208 cells derived from Madagascar periwinkle, Spi-WT cells, Spi-I-1 cells and Spi-12F cells derived from spinach, Lcy-1 cells, LcyD6 cells and LcyD7 cells derived from gourds, OS-1 cells derived from *Oryza sativa*, Vma-1 cells derived from *Vinca rosea*, PSB cells, PSW cells and PSG cells derived from sesame, and ZE3 cells derived from *Zinnia elegans*.

When cultured plant cells are used as hosts, transformation is performed by introducing a recombinant expression vector for producing hyaluronic acid into the plant by the *Agrobacterium* binary vector method, the Particle Bombardment method or the electroporation method into protoplasts, where the vector includes DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity under the control of a promoter(s) capable of functioning in plants, and then isolating the tumor tissues, shoots, hair roots and the like resulting from the transformation.

Tumor tissues, shoots, hair roots and the like obtained as described above can be directly used for cell culture, tissue culture or organ culture. These transformants can also be regenerated into plants by previously known plant tissue culture methods, by applying plant hormones in appropriate concentrations and the like.

To regenerate a plant from a cell into which a hyaluronic acid synthase gene has been introduced, such a plant cell may be cultured on a regeneration medium, a hormone-free MS medium or the like. The resultant rooting young plant can be planted in soil and grown. Methods for regeneration differ depending on the type of the plant cell, but it is possible to use a previously known method for plant tissue culture.

For example, the method of Fujimura et al. (Fujimura et al. (1955), Plant Tissue Culture Lett., Vol. 2: p. 74) can be used for *Oryza sativa*, the method of Shillito et al. (Shillito et al. (1989), Bio/Technology, Vol. 7: p. 581, and Gorden-Kamm (1990), Plant Cell, 2, 603) can be used for maize, and the method of Visser et al. (Visser et al. (1989), Theor. Appl. Genet, Vol. 78: p 589) can be used for potatoes. The method of Nagata et al. (Nagata et al. (1971), Planta 99, 12) can be

used for tobacco, and the method of Akama et al. (Akama et al. (1992), Plant Cell Rep., Vol. 12: p. 7) can be used for *Arabidopsis thaliana*.

Plants produced by such methods, or the progenies; for example, plants regenerated from seeds, stem tubers, cutting and the like) having the same nature thereof, are also objectives of the present invention.

In order to produce a protein with hyaluronic acid synthase activity and a protein having sugar-nucleotide synthase activity in plants, and further to produce and accumulate or secrete hyaluronic acid, DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity are preferably placed under the control of a promoter(s) capable of functioning in plants, so that the DNA is specifically expressed in the desired tissues or organs.

For the expression to be controlled as such, a tissue specific- or an organ-specific promoter may be further inserted into a recombinant expression vector.

If a stage-specific promoter is used instead, a target gene can be expressed only during a particular period. Therefore, productivity can be improved only during the particular period. For example, use of a vegetative stage-specific promoter improves productivity only during the vegetative stage.

Examples of organ-specific promoters are root-specific promoters, tuber-promoters tuber-specific promoters, leaf-specific promoters, seed-specific promoters, stem-specific promoters and the like.

Examples of tissue-specific promoters are green tissue-specific promoters and the like.

More specifically, usable promoters include constitutively high expression promoters such as a CaMV35S promoter, which is a promoter of the cauliflower mosaic virus 35S RNA, and the like. Green tissue-specific promoters include, for example, a rbs promoter for a gene encoding the small subunit protein of ribulose-1,5-bisphosphate carboxylase, a CAB promoter for a gene encoding the chlorophyll a/b-binding protein, and a GapA promoter for a gene encoding the A subunit protein of glyceraldehyde-3-phosphate dehydrogenase. Seed-specific promoters include a LOX promoter of the lipoxygenase gene, a Psl promoter of the lectin gene, an AmylA promoter of the amylase gene, and the like. Root-specific promoters include a A6H6H promoter of the hyoscyamine 6b-hydroxylase gene, a PMT promoter of the putrescine N-methyltransferase, and the like. Stem-specific promoters include a Sus4 promoter of the sucrose synthase, a patatin promoter for a gene encoding the glycoprotein, and the like.

It is also conceivable to control the expression of DNA encoding a protein with hyaluronic acid synthase activity and DNA encoding a protein with sugar-nucleotide synthase activity using an inducible promoter. Examples of the induction promoters are described below.

Examples of inducible promoters include a PR1a promoter, which is a promoter of a disease resistance related gene whose expression level is enhanced by injury or an addition of salicylic acid, and an rd29A promoter whose expression level is enhanced by dryness, low temperature, high salt concentration, addition of abscisic acid and the like. Examples of promoters whose expression is induced by compounds used as agricultural chemicals include a GST-27 promoter for a gene encoding a 27 KDa subunit protein of glutathion-S-transferase and is induced by herbicide safeners, a kinase promoter and a PR promoters for genes being induced by benzo(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH). In addition, in order to more stably express DNA encoding a protein with hyaluronic acid synthase activity and

DNA encoding a protein with sugar-nucleotide synthase activity in plant cells, insulators may be utilized, a signal peptide may be added to localize the a protein with hyaluronic acid synthase activity and a protein with sugar-nucleotide synthase activity in a target organelle, a part of hyaluronic acid synthase may be substituted or deleted, and the like.

The plants subjected to transformation include any plants in which gene transfer is possible.

The plants or the plant bodies of the present invention include monocotyledons and dicotyledons of angiosperms, and gymnosperms. Such plants include optionally useful plants, particularly crop plants, vegetable plants, flower plants and woody plants.

The plants or the plant bodies of the present invention also include pteridophytes and bryophytes.

Examples of plant species usable in the present invention specifically include plants belonging to the families Solanaceae, Gramineae, Cruciferae, Rosaceae, Leguminosae, Cucurbitaceae, Labiatae, Liliaceae, Chenopodiaceae, Umbelliferae, Myrtaceae, Convolvulaceae, and like.

Examples of plants belonging to Solanaceae include the plants belonging to the genus *Nicotiana*, *Solanum*, *Datura*, *Lycopersion* or *Petunia*, and, for example, include tobacco, eggplants, potatoes, tomatoes, chili peppers, petunias and the like.

Examples of plants belonging to Gramineae include the plants belonging to the genus *Oryza*, *Hordeum*, *Secale*, *Saccharum*, *Echinochloa* or *Zea*, and, for example, include *Oryza sativa*, barley, rye, cockspear, Sorghums, corn, sugarcane and the like.

Examples of plants belonging to Cruciferae include the plants belonging to the genus *Raphanus*, *Brassica*, *Arabidopsis*, *Wasabia* or *Capsella*, and, for example, include daikon radish, rapeseed, *Arabidopsis thaliana*, Japanese horseradish, Shepherd's Purse and the like.

Examples of plants belonging to Rosaceae include the plants belonging to the genus *Orunus*, *Malus*, *Pynus*, *Fragaria* or *Rosa*, and, for example, include Japanese apricots, peaches, apples, pears, strawberry, roses and the like.

Examples of plants belonging to Leguminosae include the plants belonging to the genus *Glycine*, *Vigna*, *PHASEOLUS*, *Pisum*, *Vicia*, *Arachis*, *Trifolium*, *Alfalfa* or *Medicag*, and, for example, include soy beans, adzuki beans, butter beans, peas, fava beans, peanuts, clovers, bur clovers and the like.

Examples of plants belonging to Cucurbitaceae include the plants belonging to the genus *Luffa*, *Cucurbita* or *Cucumis*, and, for example, include gourds, pumpkins, cucumber, melons and the like.

Examples of plants belonging to Labiatae include the plants belonging to the genus *Lavandula*, *Mentha* or *Perilla*, and, for example, include lavender, mint, Japanese basil and the like.

Examples of plants belonging to Liliaceae include the plants belonging to the genus *Allium*, *Lilium* or *Tulipa*, and, for example, include Welsh onions, garlic, lilies, tulips and the like.

Examples of plants belonging to Chenopodiaceae include the plants belonging to the genus *Spinacia*, and, for example, include sugar beets, spinach and the like.

Examples of plants belonging to Umbelliferae include the plants belonging to the genus *Angelica*, *Daucus*, *Cryptotaenia* or *Apitum*, and, for example, include shishiudos, carrots, hornworts, celeries and the like.

Examples of plants belonging to Convolvulaceae include the plants belonging to the genus *Ipomoea*, and, for example, include sweet potatoes and the like.

The progenies having the same nature as the above transgenic plants, or the organs or tissues thereof are also the subjects of the present invention.

The transgenic plant cells which produce a protein with hyaluronic acid synthase activity and a protein having a sugar-nucleotide synthase activity are included in the present invention. The transgenic plants which produce a protein having hyaluronic acid synthase activity and a protein having sugar-nucleotide synthase activity, the progenies having the same nature thereof, or the organs or tissues thereof, are also included.

Extraction of Hyaluronic Acid

Below is an example of methods for isolating or obtaining hyaluronic acid by co-expressing a protein with hyaluronic acid synthase activity and an exogenous protein having an activity of synthesizing the sugar nucleotide, and extracting hyaluronic acid from the transgenic plant cells or the transgenic plants that have acquired the ability to produce hyaluronic acid, progenies, or organs or tissues thereof having the same nature thereof.

The transgenic plant cells or the transgenic plants are cultured or grown, the hyaluronic acid is produced, and subsequently, the hyaluronic acid is optionally extracted from the transgenic plant cells or the transgenic plants by a known method. The extracts are checked for hyaluronic acid.

For example, the transgenic plants, the progenies having the same nature thereof, the organs or the tissues thereof, or the like can be subsequently dried, grinded and then extracted by an appropriate organic solvent. The extract containing the hyaluronic acid is filtrated, and a filtrate containing hyaluronic acid and no plant cells is obtained. This filtrate is purified by diafiltration to remove low-molecular-weight impurities. It is possible to separate the hyaluronic acid by the diafiltration of the filtrate containing dissolved hyaluronic acid with pure water followed by continuously discarding the filtrate. When hyaluronic acid is used in pharmaceuticals, a step of precipitating nucleic acids from the solution may be further performed. This step can be, for example, performed by adding cation surfactant such as quaternary ammonium compounds of cetylpyridinium chloride.

Hyaluronic acid accumulated in the transformed plant cells may be also purified by known methods for the separation.

Use of Hyaluronic Acid

Hyaluronic acid acquired by the present invention can be usefully utilized for cosmetic and pharmaceutical compositions, or biomaterials. Specifically, hyaluronic acid can be used as a moisturizing composition in cosmetics, a therapeutic agent for arthritis, chronic rheumatism, burns and cuts, or a component in eye drops.

Hyaluronic acid obtained by the production method of the present invention may be used as an active agent to make cosmetic compositions. For example, hyaluronic acid can be applied in liquid forms such as aqueous solutions, oil solutions, emulsions and suspensions, in semi-solid forms such as gels and creams, and in solid forms such as powders, granules, capsules, microcapsules and solids. Hyaluronic acid can be prepared into those forms using known methods, and made in the formulation of lotions, emulsions, gels, creams, ointments, emplastrums, cataplasms, aerosols, suppositories, injections, powders, granules, tablets, pills, syrups, troches and the like. Such formulations can be applied by applying, attaching, spraying, drinking and the like. Particularly among those formulations, lotions, emulsions, creams, ointments, emplastrums, cataplasms, aerosols and the like are suitable for skin applications. For cosmetics, hyaluronic acid can be used for skin care cosmetics such as lotions, serums, emulsions, creams and masks, makeup cosmetics such as makeup

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base lotions, makeup creams, foundations in emulsions, cream and ointment forms, lipsticks, eye shadows and cheek colors, body care cosmetics such as hand creams, leg creams, body lotions and the like, bath essences, oral care cosmetics and hair care cosmetics. Hyaluronic acid can be produced into such formulations according to the general method for making cosmetics.

EXAMPLES

The following Examples illustrate the present invention in further detail, but are not intended to limit the scope of the invention.

Example 1

Isolation of *Arabidopsis-thaliana*-Derived UGD Gene

A UDP-glucose dehydrogenase (BT006380:AtUGD1, SEQ ID NO: 15) gene has been isolated from *Arabidopsis thaliana* and shown to have activity (Plant J. 2000 21(6):537-46). Further, the NCBI BLAST (www.ncbi.nlm.nih.gov/BLAST/) database shows three types of *Arabidopsis-thaliana*-derived genes that are predicted to encode UGD (AF424576:AtUGD2 (SEQ ID NO: 17), AY056200:AtUGD3 (SEQ ID NO: 19), and AY070758:AtUGD4 (SEQ ID NO: 21)). These four types of UGD genes were cloned to confirm their UGD activity.

RNA was extracted from *Arabidopsis thaliana* according to the RNeasy (QIAGEN) protocol. For a reverse transcription reaction, 2 µg of total RNA was dissolved in 5.5 µL of sterile water, mixed with 1 µL of 10 µM oligo d(T) primer, and thermally denatured at 70° C. for 10 minutes. After rapid cooling, the reverse transcription reaction was performed using a ReverTraAce kit (Toyobo), at 42° C. for 30 minutes and at 99° C. for 5 minutes.

For PCR amplification of the *Arabidopsis-thaliana*-derived UGD genes, PCR primers were designed based on the four nucleotide sequences on the database. Restriction enzyme cleavage sites that are necessary for introduction into the expression vector pMAL-c2 (NewEngland Biolab) were added to the primers. EcoRI or HindIII (SEQ ID NO: 23 or 24) were added to AtUGD1; EcoRI or PstI (SEQ ID NO: 25 or 26) to AtUGD2; EcoRI or PstI (SEQ ID NO: 27 or 28) to AtUGD3; and EcoRI or XbaI (SEQ ID NO: 29 or 30) to AtUGD4. PCR was performed using a KOD-plus-DNA polymerase (Toyobo) and a reaction program of 1 cycle of 94° C. for 2 minutes, 3 cycles of 94° C. for 15 seconds, 45° C. for 30 seconds, and 68° C. for 1 minute, and 30 cycles of 94° C. for 15 seconds, 55° C. for 30 seconds, and 68° C. for 1 minute. One microliter of reverse transcription reaction product was used as template DNA. An agarose gel electrophoresis analysis showed PCR-amplified bands with predicted size. Each PCR-amplified fragment was cleaved with restriction enzymes (AtUGD1: EcoRI and HindIII; AtUGD2 and AtUGD3: EcoRI and PstI; AtUGD4: EcoRI and XbaI), and cloned into pMAL-c2 digested with the same restriction enzymes, using Ligation High (Toyobo). Using the ligation mixture, *Escherichia coli* strain JM109 was transformed by the above-mentioned known method, and the transformants were applied to Luria-Bertani (LB) agar medium (10 g/L bactotryptone, 10 g/L yeast extract, 5 g/L sodium chloride, 15 g/L agar) containing ampicillin (50 µg/mL), and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of transformants using a known method. The nucleotide sequences of the inserted fragments were determined

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using a DNA sequencer, and it was confirmed that the amplified genes were those represented by SEQ ID NOS: 15, 17, 19, and 21. Thus, the plasmids pMAL-c2/AtUGD1, pMAL-c2/AtUGD2, pMAL-c2/AtUGD3, and pMAL-c2/AtUGD4 were constructed.

The above demonstrates that, in addition to AtUGD1, which has already been reported, three types of UGD genes are expressed in *Arabidopsis thaliana*.

Escherichia coli strain JM109 carrying the above expression plasmids were cultured overnight in LB liquid medium containing ampicillin (50 µg/mL) at 37° C. LB medium (30 ml) containing ampicillin (50 µg/mL) and 0.2% glucose was inoculated with 300 µL of the preculture and cultured at 37° C. for 2 hours. The temperature was then lowered to 25° C., isopropyl-β-thiogalactopyranoside (IPTG) at a final concentration of 0.3 mM was added, and expression of the recombinant proteins was induced for 24 hours.

Cells were recovered from 1 mL of the culture medium by centrifugation and disrupted by ultrasonic disintegration to prepare a crude extract. MBP fusion proteins were purified using MagExtractor-MBP-(Toyobo).

FIG. 1 shows the SDS-PAGE analysis of the expressed MBP fusion proteins. A band of the predicted size was observed in all enzyme solutions of the clones.

The UGD activity of the obtained MBP fusion proteins was measured according to the method reported by Tenhaken and Thulke (Plant Physiol. 1996, 112:1127-34). In the method, the increase in NADH caused by UGD reaction is detected as an increase in Abs340. Specifically, 15 µL of the enzyme solution (MBP-UGD fusion protein) was added to a reaction mixture (pH 8.0) containing UDP-glucose (4 mM), NAD⁺ (1 mM), EDTA (1 mM), and Tris-HCl (20 mM); a reaction was carried out at 37° C.; and the absorbance (Abs340) of the reaction mixture was measured over time. Table 1 shows the enzymatic activity of the MBP fusion proteins.

TABLE 1

	U/mg
AtUGD1	2.15
AtUGD2	1.32
AtUGD3	1.15
AtUGD4	0.25
cvUGD-HI	0.06
cvUGD0KA	0.14

The results demonstrate that all enzyme solutions of AtUGD1, AtUGD2, AtUGD3, and AtUGD4 have UGD activity, indicating that, in *Arabidopsis thaliana*, the AtUGD2, AtUGD3, and AtUGD4 genes, as well as the already reported AtUGD1 gene, all encode UGD.

Example 2

Isolation of Chlorella-Virus-Derived UGD Gene

For isolation of the chlorella-virus-derived UDP-glucose dehydrogenase (cvUGD) gene by PCR, the primers of SEQ ID NOS: 31 and 32 were designed based on the known sequence information of chlorella virus strain PBCV-1. For introduction into the expression vector pMAL-c2, EcoRI and PstI sites were added to the 5'-end and 3'-end primers, respectively. PCR was carried out using a KOD-plus-DNA polymerase and a reaction program of 1 cycle of 94° C. for 2 minutes and 30 cycles of 94° C. for 15 seconds, 60° C. for 30 seconds, and 68° C. for 1 minute. The genomic DNA of chlorella virus strain Hirosaki (CVHI1) and strain Kakuno-

date (CVKA1) was used as template DNA. An agarose gel electrophoresis analysis showed PCR-amplified bands with predicted size. Each PCR-amplified fragment was cleaved with EcoRI and PstI, and cloned into pMAL-c2 digested with the same restriction enzymes, using Ligation High. Using the ligation mixture, *Escherichia coli* strain JM109 was transformed according to the above-mentioned known method, and the transformants were applied to LB agar medium containing 50 µg/mL ampicillin, and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of the transformants using a known method.

The nucleotide sequences of the inserted fragments were determined using a DNA sequencer, and novel UGD genes derived from strains CVHI1 and CVKA1 were obtained and named “cvUGD-HI gene” and “cvUGD-KA gene”. The nucleotide sequences of the cvGFAT-HI and cvUGD-KA genes are shown in SEQ ID NOS: 11 and 13. Thus, the plasmids pMAL-c2/cvUGD-HI and pMAL-c2/cvUGD-KA were constructed.

MBP fusion proteins were expressed and purified in the same manner as in Example 1. FIG. 2 shows the SDS-PAGE analysis of the obtained MBP fusion proteins. A band of the predicted size was observed in all enzyme solutions of the clones.

The UGD activities of the expressed MBP fusion proteins were measured in the same manner as in Example 1. Table 1 shows the enzymatic activity of the MBP fusion proteins.

The UGD activity measurement by the above method demonstrated that both cvUGD-HI and cvUGD-KA encode functional enzymes.

Example 3

Isolation of *Arabidopsis-thaliana*-Derived GFAT Gene

As a plant-derived GFAT gene, a corns-derived GFAT gene (Accession No. AY106905) has been reported to be isolated (WO 00/11192), but no GFAT genes have been isolated from other species of plants. The NCBI BLAST (www.ncbi.nlm.nih.gov/BLAST/) database shows an *Arabidopsis-thaliana*-derived GFAT gene (Accession No. NM_113314), which is highly homologous to the known gene. The nucleotide sequence of the *Arabidopsis-thaliana*-derived GFAT gene is shown in SEQ ID NO: 9 in the Sequence Listing.

RNA was extracted from *Arabidopsis thaliana* according to the RNeasy (QIAGEN) protocol. For a reverse transcription reaction, 2 µg of total RNA was dissolved in 5.5 µL of sterile water, mixed with 1 µL of 10 µM oligo d(T) primer, and thermally denatured at 70° C. for 10 minutes. After rapid cooling, the reverse transcription reaction was performed using a ReverTraAce kit (Toyobo), at 42° C. for 30 minutes and at 99° C. for 5 minutes.

For PCR amplification of the *Arabidopsis-thaliana*-derived GFAT gene, the primers of SEQ ID NOS: 33 and 34 were designed based on nucleotide sequences on the database. For introduction into a cell-free expression vector pEU-NII (Toyobo), a Sall site was added to the 3'-end primer. PCR was performed using a KOD-plus-DNA polymerase and a reaction program of 1 cycle of 94° C. for 2 minutes, 3 cycles of 94° C. for 15 seconds, 45° C. for 30 seconds, and 68° C. for 1 minute, and 30 cycles of 94° C. for 15 seconds, 55° C. for 30 seconds, and 68° C. for 1 minute. One microliter of reverse transcription reaction product was used as template DNA. An agarose gel electrophoresis analysis showed PCR-amplified bands with predicted size. The PCR-amplified fragment was cleaved with a restriction enzyme Sall, and cloned into pEU-

NII digested with restriction enzymes EcoRV and Sall, using Ligation High. Using the ligation mixture, *Escherichia coli* strain JM109 was transformed according to the above-mentioned known method, and the transformants were applied to LB agar medium containing 50 µg/mL ampicillin, and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of the transformants using a known method. Thus, the plasmid pEU-NII/AtGFAT was constructed.

The nucleotide sequence of the inserted fragment was determined using a DNA sequencer, and it was confirmed that the amplified fragment was the gene represented by SEQ ID NO: 9. The above reveals that the GFAT gene is expressed in *Arabidopsis thaliana*.

Using PROTEIOS (a registered trademark of Toyobo), the recombinant protein of the AtGFAT was expressed. Specifically, a reaction was carried out at 37° C. for 4 hours using 5 µg of the plasmid pEU/AtGFAT as a template and a T7 RNA Polymerase, to synthesize mRNA. Thereafter, 6 µg of mRNA was mixed with wheat germ extract, and a reaction was carried out at 26° C. for 24 hours by the bilayer method. The reaction mixture was suspended in sample buffer (50 mM Tris-HCl (pH6.8), 2% SDS, 10% glycerol, 0.6% β-mercaptoethanol) and boiled for 5 minutes, and then the expressed protein was analyzed by SDS-PAGE. A band of the predicted size (about 75 kDa) was observed, indicating the expression of the AtGFAT protein (FIG. 3).

The protein expression solution was subjected to GFAT reaction. Specifically, 50 µL of the protein expression solution was added to a solution (pH 7.0) containing fructose-6-phosphate (15 mM), L-glutamine (15 mM), EDTA (1 mM), DTT (1 mM), and KH₂PO₄ (60 mM), and reacted at 37° C. for 4 hours. Glucosamine-6-phosphate in the reaction mixture was measured using the Reissig method (J. Biol. Chem, 1955, 217 (2), 959-66), which is an improvement of the Morgan & Elson method, to evaluate GFAT activity. FIG. 4 shows the results. Glucosamine-6-phosphate was not detected in pEU-NII/DHFR used as a control, whereas an increase of glucosamine-6-phosphate was observed in pEU-NII/AtGFAT, demonstrating that an active GFAT enzyme was present in the protein expression solution.

The above reveals that the AtGFAT gene encodes a functional enzyme in *Arabidopsis thaliana*.

Example 4

Isolation of Chlorella-Virus-Derived GFAT Gene

PCR primers were prepared to isolate the chlorella-virus-derived glutamine-fructose-6-phosphate amidotransferase gene (cvGFAT) by PCR. Based on already reported nucleotide sequence information of the chlorella virus strain PBCV-1, the primers of SEQ ID NOS: 35 and 36 were designed so as to amplify from 100 bp outside the putative GFAT region PCR was performed using a KOD-plus-DNA polymerase and a reaction program of 1 cycle of 94° C. for 2 minutes and 30 cycles of 94° C. for 15 seconds, 60° C. for 30 seconds, and 68° C. for 1 minute. The genomic DNA of chlorella virus strains Hirosaki (CVHI1) and Kakunodate (CVKA1) was used as template DNA. The PCR-amplified fragments were cloned into a pPCR-Script Amp SK(+) cloning vector (Stratagene). The nucleotide sequences of the inserted fragments were determined using a DNA sequencer, and novel GFAT genes derived from strains cvHI1 and cvKA1 were identified and named “cvGFAT-HI gene” and “cvGFAT-KA gene”. The nucleotide sequences of cvGFAT-HI and cvGFAT-KA genes are shown in SEQ ID NOS: 5 and 7.

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The open reading frame regions of the cvGFAT-HI and cvGFAT-KA genes represented by SEQ ID NOS: 5 and 7 were cloned into vectors for cell-free protein synthesis to express proteins.

PCR was performed under the above-mentioned conditions using pPCR-Script Amp SK(+) cloning vector containing the cvGFAT-HI gene as a template and the primers represented by SEQ ID NOS: 37 and 38. PCR was performed under the above-mentioned conditions using pPCR-Script Amp SK(+) cloning vector containing the cvGFAT-KA gene as a template and the primers represented by SEQ ID NOS: 37 and 39. For the PCR, a KOD-plus-DNA polymerase, and a reaction cycle of 1 cycle of 94° C. for 2 minutes and 30 cycles of 94° C. for 15 seconds, 60° C. for 30 seconds, and 68° C. for 1 minute, were used. Each PCR-amplified fragment was cleaved with XbaI and cloned into the EcoRV and XbaI sites of the PEU-NII vector. Using the ligation mixture, *Escherichia coli* strain DH5 α was transformed according to the above-mentioned known method, and the transformants were applied to LB agar medium containing 50 μ g/mL ampicillin, and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of the transformants using a known method. Thus, the plasmids pEU-NII/cvGFAT-HI and pEU-NII/cvGFAT-KA were constructed.

Using pEU-NII/cvGFAT-HI and pEU-NII/cvGFAT-KA and PROTEIOS (registered trademark), proteins were expressed in the same manner as in Example 3. SDS-PAGE analysis of the expressed proteins confirmed bands of the predicted sizes (about 65 kDa), indicating the expression of cvGFAT proteins (FIG. 3).

The protein expression solution was subjected to GFAT reaction in the same manner as in Example 3. FIG. 4 shows the results. Glucosamine-6-phosphate was not detected in pEU/DHFR used as a control, whereas an increase of glucosamine-6-phosphate was observed in pEU/cvGFAT-HI, confirming that an active GFAT enzyme was present in the protein expression solution.

Example 5

Cloning of Chlorella-Virus-Derived HAS Gene into pBI121

For the cloning of a chlorella-virus-derived hyaluronic acid synthetase gene (cvHAS, SEQ ID NO: 1) into the plant transformation vector (hereinafter also referred to as "expression vector") pBI121 (Jefferson et al., 1987, EMBO J, 6, 3901-3907), the primers represented by SEQ ID NOS: 40 and 41 were prepared. PCR was performed using cvHAS-containing plasmid DNA as a template, a KOD-plus-DNA polymerase, and a reaction program of 1 cycle of 94° C. for 2 minutes, 3 cycles of 94° C. for 15 seconds, 45° C. for 30 seconds, and 68° C. for 1 minute, and 30 cycles of 94° C. for 15 seconds, 55° C. for 30 seconds, and 68° C. for 1 minute. PCR-amplified fragment was purified and cleaved with BamHI and DraI. Subsequently, cvHAS was inserted into the expression vector pBI121 as follows: pBI121 was digested with the restriction enzyme SacI, blunted with Blunting High (Toyobo), and digested with BamHI; and then the cvHAS gene digested with a restriction enzyme was cloned. Using the ligation mixture, *Escherichia coli* strain DH5 α was transformed according to the above-mentioned known method, and the transformants were applied to LB agar medium containing 50 μ g/mL ampicillin, and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of the transfor-

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mants using a known method. Thus, pBI121/cvHAS (hereinafter sometimes referred to as pBIHA) containing cvHAS was prepared.

Example 6

Cloning of Chlorella-Virus-Derived GFAT Gene into pBI121

PCR was performed using the primers represented by SEQ ID NOS: 42 and 38 and cvGFAT-HI-containing plasmid DNA as a template. For the PCR, KOD-plus- and a reaction program of 1 cycle of 94° C. for 2 minutes, 2 cycles of 94° C. for 15 seconds, 50° C. for 30 seconds, and 68° C. for 1 minute, and 28 cycles of 94° C. for 15 seconds, 60° C. for 30 seconds, and 68° C. for 1 minute, were used. The PCR-amplified fragment was digested with BamHI. Subsequently, cvGFAT-HI was inserted into the expression vector ppBI121 as follows: pBI121 was digested with SacI, blunted with Blunting High, and digested with BamHI, and then the cvGFAT-HI gene digested with the above restriction enzymes was cloned. Using the ligation mixture, *Escherichia coli* strain DH5 α was transformed according to the above-mentioned known method, and the transformants were applied to LB agar medium containing 50 μ g/mL ampicillin and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of the transformants using a known method. Thus, pBI121/cvGFAT-HI (hereinafter sometimes referred to as pBIGF) containing cvGFAT-HI was prepared.

Example 7

Subcloning of Chlorella-Virus-Derived GFAT Gene and HAS Gene into pBluescript

pBIHA was digested with the restriction enzymes PvuII and PstI, in that order, and subcloned into the PstI and SmaI sites of pBluescript to obtain pBluescript/35S-cvHAS-NOS (hereinafter sometimes referred to as pBSHA).

pBIGF was digested with the restriction enzymes PvuII and SphI, in that order. After blunting with Blunting High (Toyobo), pBIGF was subcloned into the EcoRV site of pBluescript to obtain pBluescript/35S-cvGFAT-NOS (hereinafter sometimes referred to as pBSGF).

pBSGF was digested with the restriction enzymes KpnI and NotI, in that order. After blunting with Blunting High (Toyobo), 35S-cvGFAT-NOS was subcloned into pBSHA previously digested with SpeI, blunting, and dephosphorylation, in that order, to obtain pBluescript/cvHAS-cvGFAT (hereinafter sometimes referred to as pBSHG).

Example 8

Cloning of Chlorella-Virus-Derived GFAT Gene and HAS Gene into pBI121

pBSHG was digested with the restriction enzymes KpnI and NotI in that order, and the expression cassette of cvHAS-cvGFAT was cleaved and blunted. Then, cvHAS-cvGFAT was inserted into the expression vector pBI121 as follows: pBI121 was digested with the restriction enzymes SacI and HindIII, and blunted with Blunting High, and then the cvHAS-cvGFAT gene digested with the above restriction enzymes was cloned. Using the ligation mixture, *Escherichia coli* strain DH5 α was transformed according to the above-mentioned known method, and the transformants were applied to LB agar medium containing 50 μ g/mL ampicillin,

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and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of the transformants using a known method. Thus, pBI121/cvHAS-cvGFAT (hereinafter sometimes referred to as pBIHG) containing cvHAS-cvGFAT was prepared.

Example 9

Preparation of Electrocompetent Cells

Five milliliters of LB medium was inoculated with a single colony of *Agrobacterium* LBA4404 (*Agrobacterium tumefaciens* strain LBA4404), and subjected to shaking culture overnight at 28° C. The culture medium was inoculated into 500 mL of LB medium and subjected to shaking culture at 28° C. until the turbidity at 600 nm became 0.5. The culture medium was harvested by centrifugation (5000 rpm, 10 min, 4° C.); the supernatant was removed; 500 mL of sterile water was added to suspend and wash the cells; centrifugation (5000 rpm, 10 min, 4° C.) was carried out again to harvest the cells; and the supernatant was removed. After performing the above procedure twice, the precipitates were suspended in 20 mL of cooled 10% glycerol solution, the cells were harvested by centrifugation (5000 rpm, 10 min, 4° C.), and the supernatant was removed. The precipitates were suspended in 3 mL of cooled 10% glycerol solution, and 40- μ L aliquots of the suspension were placed in 1.5 mL centrifuge tubes, frozen with liquid nitrogen, and stored at -80° C.

Example 10

Introduction of pBIHG into *Agrobacterium* Strain LBA4404

A suspension obtained by mixing 1 μ L of the expression plasmid pBIHG (200 μ g/ml) with 40 μ L of electrocompetent cells of *A. tumefaciens* LBA4404 (Invitrogen) was poured into a cuvette (distance between the electrodes: 1 mm) previously cooled on ice, and a pulsed electric field (1.8 kV, 25 μ F, 200 Ω) was applied. Immediately thereafter, 500 μ L of SOC was added, and the resulting mixture was incubated at 28° C. for 3 hours. The incubated cells were then applied to LB plate medium containing kanamycin, and cultured at 25° C. for 3 days to obtain *Agrobacterium* cells carrying pBIHG.

Example 11

Infection of Tobacco with *Agrobacterium tumefaciens* Strain LBA4404 Containing pBIHG

Tobacco (*Nicotiana tabacum* SR-1) was transformed according to the leaf disc method using *Agrobacterium* ("Plant Biotechnology II" edited by Yasuyuki Yamada and Yoshimi Okada, Tokyo Kagaku Dojin, 1991). Tobacco leaf discs were immersed for 3 minutes in an *Agrobacterium* culture medium carrying pBIHG or pBIHA previously cultured overnight at 28° C. in 5 mL of LB medium containing 50 mg/L kanamycin. Excess cells were then removed on filter paper. The leaf discs were placed in a differentiation medium prepared by adding 3% sucrose, B5 vitamin, 1 mg/L benzylaminopurine, 1 mg/L naphthalene acetic acid, and 0.3% gellan gum to MS (Murashige and Skoog) inorganic salt (Murashige and Skoog, 1962, *Physiol. Plant.*, 15, 473) and adjusting the pH to 5.7, and were left to stand in the dark at 28° C. for 2 days. The infected leaf discs were washed three times with sterile water, and excess moisture was removed on filter paper. The leaf discs were then left to stand in the differen-

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tiation medium containing kanamycin (100 mg/L) and cefotaxime (250 mg/L) as antibiotics, and callus formation was induced at 25° C. under 16-hour light conditions. Three weeks after starting the induction, morphologically normal shoots were selected, cut out in a form containing stems and leaves, and transferred into a rooting medium (MS inorganic salt, 3% sucrose, B5 vitamin and 0.3% gellan gum, pH 5.7) containing kanamycin (100 mg/L) and cefotaxime (250 mg/L) to induce rooting at 25° C. under 16-hour light conditions. After two weeks, rooted shoots were transferred to a fresh rooting culture medium to obtain lines with growing stems and leaves.

Example 12

Quantitation of Hyaluronic Acid Produced by Transformed Tobacco

About 100 mg of transformed tobacco leaves obtained by the infection with *Agrobacterium* described above was transferred to a 2 mL tube, and suspended in 200 μ L of buffer (containing 20 mM Tris-HCl at pH 7.5, 0.2M NaCl, 1 mM EDTA, and 10 mM 2-ME), and 400 mg of stainless steel beads (diameter: 4.8 mm) were added. The tobacco leaves were pulverized by shaking and agitating the tube using Bead Smash (Wakenyaku, BS-12) (4,000 rpm, 2 minutes). The liquid after pulverization was centrifuged (15,000 rpm, 10 minutes), and the supernatant was recovered as a crude extract. The crude extract was diluted with water and used as a measurement sample. The quantitation of hyaluronic acid was performed using a hyaluronic acid plate "Chugai" (Fujirebio, Inc.). FIG. 5 shows the results. The transformed tobacco into which the cvHAS-cvGFAT gene had been introduced had significantly improved hyaluronic acid productivity compared to the transformed tobacco into which the cvHAS gene had been introduced.

Example 13

Cloning of Chlorella-Virus-Derived UGD Gene into pBI121

PCR was performed using the primers represented by SEQ ID NO: 43 and SEQ ID NO: 44 and plasmid DNA containing cvUGD-HI as a template. For the PCR, KOD-plus-, and a reaction program of 1 cycle of 94° C. for 2 minutes, 2 cycles of 94° C. for 15 seconds, 50° C. for 30 seconds, and 68° C. for 1 minute), and 28 cycles of 94° C. for 15 seconds, 60° C. for 30 seconds, and 68° C. for 1 minute, were used. PCR-amplified fragment was digested with BamHI and SacI. Subsequently, cvUGD-HI was inserted into the expression vector pBI121 as follows: pBI121 was digested with SacI and then with BamHI, and the cvUGD-HI gene digested with the above-mentioned restriction enzymes was cloned into pBI121. Using the ligation mixture, *Escherichia coli* strain DH5 α was transformed according to the above-mentioned known method, and the transformant was applied to LB agar medium containing 50 μ g/ml ampicillin, and cultured overnight at 37° C. The plasmid was extracted from the grown colonies of the transformants using a known method. Thus, pBIUG containing cvUGD-HI was prepared.

Example 14

Subcloning of Chlorella-Virus-Derived UGD Gene into pBluescript

pBIUG was digested with restriction enzymes EcoRI and HindIII, subcloned into the EcoRI and HindIII sites of pBluescript to prepare pBSUG.

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pBSUG was digested with SpeI and blunted with Blunting High to destroy the SpeI site. Subsequently, pBSHA was digested with NotI, blunted, and digested with KpnI to cut out 35S-cvHAS-NOS. pBSUG was digested with XhoI, blunted, and digested with KpnI; and 35S-cvHAS-NOS, which had been cut out, was ligated to produce pBSHU. pBSGF was digested with Sall, blunted, and digested with NotI to cut out 35S-cvGFAT-NOS. pBHU was digested with SpeI, blunted, and digested with NotI, and 35S-cvGFAT-NOS, which had been cut out, was ligated to produce pBSHUG.

Example 15

Cloning of Chlorella-Virus-Derived UGD Gene, GFAT Gene, and HAS Gene, into pBI121

Using synthetic DNA, modified pBI121 was produced in which a SmaI site had been added downstream of the HindIII site of pBI121, and a KpnI site had been added upstream of the EcoRI site. pBSHUG was digested with NotI, blunted, and digested with KpnI; the linked expression cassettes of HAS, UGD and GFAT were cut out and cloned into the modified pBI121 cleaved with SmaI and KpnI. Using the ligation mixture, *Escherichia coli* strain DH5 α was transformed according to the above-mentioned known method, and the transformants were applied to LB agar medium containing 50 μ g/ml ampicillin, and cultured overnight at 37 $^{\circ}$ C. The plasmid was extracted from the grown colonies of the transformants using a known method. Thus, pBIHUG containing expression cassettes of HAS, UGD and GFAT were prepared.

Example 16

Preparation of Transformed Tobacco into which pBIHUG had been Introduced, and Quantitative Analysis of Hyaluronic Acid

Using the expression plasmid pBIHUG and following the procedures shown in Examples 10 and 11, pBIHUG was

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introduced into *Agrobacterium* strain LBA4404, and tobacco leaf discs were infected with *Agrobacterium* strain LBA4404 containing pBIHUG. As a result, 20 lines of tobacco were obtained in which introduction of HAS, UGD, and GFAT genes had been confirmed. Following the procedure shown in Example 12, crude extracts were prepared and hyaluronic acid was quantitated. FIG. 6 shows the results. The transformed tobacco into which the HAS, UGD and GFAT genes had been introduced showed significantly improved hyaluronic acid productivity compared to the transformed tobacco into which the cvHAS gene had been introduced and the transformed tobacco into which the cvHAS-cvGFAT gene had been introduced.

Example 17

Quantitative Analysis of Hyaluronic Acid Produced by Transformed Tobacco (T1 Generation)

Seeds were collected from the transformed tobacco of Example 12, into which the cvHAS-cvGFAT gene had been introduced, and inoculated into MS differentiation medium containing kanamycin (100 mg/L). A crude extract was obtained from the grown transformed tobacco (T1 generation) in the same manner as in Example 12, and hyaluronic acid was quantitated, demonstrating the production level of hyaluronic acid equivalent to that of T0 generation.

INDUSTRIAL APPLICABILITY

According to the present invention, a hyaluronic acid synthetase gene is expressed in a plant, and in particular, hyaluronic acid is produced in a high yield in a plant. The present invention provides a plant or cultured plant cells that are capable of producing hyaluronic acid in a higher yield than the prior art; a method for producing the plant or cultured plant cells; and an expression vector. Since safe hyaluronic acid produced in a plant can be provided at a low cost, the present invention is expected to greatly contribute to the industry.

SEQUENCE LISTING

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<212> TYPE: DNA

<213> ORGANISM: Chlorella virus

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<211> LENGTH: 568

<212> TYPE: PRT

<213> ORGANISM: Chlorella virus

<400> SEQUENCE: 2

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<212> TYPE: DNA

<213> ORGANISM: Chlorella virus

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<212> TYPE: PRT

<213> ORGANISM: Chlorella virus

<400> SEQUENCE: 4

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 515 520 525
 Thr Ile Val Leu Val Ile Tyr Phe Thr Gly Lys Ile Thr Thr Trp Asn
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 atgtccattt acgatcctga tggaaatgaa gtggaatatg aagtagagga cgttgaaatg 720
 gaacaaacta gtatgtctct tgataacttt gatcattaca tgattaagga aattaatgag 780
 caaccaatca gtatcctaaa cactataaaa aataaagggt tttatgcaga aatattcggc 840
 gatttggcgc atgaaatctt ccaaaaaata gacaacatcc tgatactggc ttgtggtaca 900
 agttatcacg ccggtcttgt aggaaaacag tggatagaga ccattgagag aatccccgtg 960
 gatggtcaca tcgagagcga atacgaacct acaattccga gagcgaacac attggtaatc 1020
 actatttcac agtcgggtga aactgaggac acgatagcgg ctttgcaacg ggcccaaac 1080
 gccgggatga tttatacatt gtgtatttgc aattcaccia agagcactct tgttcgagag 1140
 agcgttatga agtacataac gaaatgtggg tctgaggtgt cagtagcatc aacgaaggcg 1200
 tttacctcgc aactcgtagt actgtacatg ctggcaaacg tattggcaaa taaaaccgat 1260
 gatttgcctg gagacctccc acaggcaata gaacgggtga tttgttgac aatgacgaa 1320
 atgaaacact gggcggagca aatctgcaat gcgaaatctg cgatcttctt gggaagagga 1380
 ctaaacgcac cagttgcctt tgagggagcg ctgaagctca aagaaatctc ttacattcat 1440
 gcagagggct tcctgggagg tgagttgaaa catggcccc tcgcactcct tgatgacaaa 1500
 attcctgtta tcgtaaccgt agcagatcat gcttatttgg accatatcaa agcaaatatt 1560
 gacgaagtgc ttgcgaggaa cgttacggta tacgcatag tagaccagta tgtgaacatc 1620

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gagccccagg aacgccttcg cgttgtcaag gttccgtttg tatccaaaga attttctccg 1680
ataattcaca ctatcccgat gcaactgctt tcgtattacg tggcaattaa gcttggaag 1740
aacgttgaca aaccaaggaa tcttgcaaaa tccgtgacca ccttttaa 1788

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<210> SEQ ID NO 6
<211> LENGTH: 595
<212> TYPE: PRT
<213> ORGANISM: Chlorella virus

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<400> SEQUENCE: 6

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Met Cys Gly Ile Phe Gly Ala Val Ser Asn Asn Asn Ser Ile Glu Val
1          5          10          15
Ser Ile Lys Gly Ile Gln Lys Leu Glu Tyr Arg Gly Tyr Asp Ser Cys
          20          25          30
Gly Ile Ala Tyr Thr Asp Gly Gly Ala Ile Glu Arg Ile Arg Ser Val
          35          40          45
Asp Gly Ile Asp Asp Leu Arg Lys Lys Thr Ile Thr Glu Ser Ser Pro
          50          55          60
Val Ala Ile Ala His Ser Arg Trp Ser Thr Thr Gly Ile Pro Ser Val
          65          70          75          80
Val Asn Ala His Pro His Ile Ser Arg Gly Thr Ser Gly Cys Glu Ser
          85          90          95
Arg Ile Ala Val Val His Asn Gly Ile Ile Glu Asn Tyr Gln Gln Ile
          100          105          110
Arg Lys Tyr Leu Ile Asn Leu Gly Tyr Thr Phe Asp Ser Gln Thr Asp
          115          120          125
Thr Glu Val Ile Ala His Leu Ile Asp Ser Gln Tyr Asn Gly Asn Ile
          130          135          140
Leu His Thr Val Gln Met Ala Val Lys His Leu Lys Gly Ser Tyr Ala
          145          150          155          160
Ile Ala Val Met Cys His Lys Glu Ser Gly Lys Ile Val Val Ala Lys
          165          170          175
Gln Lys Ser Pro Leu Val Leu Gly Ile Gly Ser Asp Gly Ala Tyr Tyr
          180          185          190
Ile Ala Ser Asp Val Leu Ala Leu Pro Thr Asn Lys Val Val Tyr Ile
          195          200          205
Ser Asp Gly Phe Ser Ala Glu Leu Ser Pro Gly Ser Met Ser Ile Tyr
          210          215          220
Asp Pro Asp Gly Asn Glu Val Glu Tyr Glu Val Glu Asp Val Glu Met
          225          230          235          240
Glu Gln Thr Ser Met Ser Leu Asp Asn Phe Asp His Tyr Met Ile Lys
          245          250          255
Glu Ile Asn Glu Gln Pro Ile Ser Ile Leu Asn Thr Ile Lys Asn Lys
          260          265          270
Gly Phe Tyr Ala Glu Ile Phe Gly Asp Leu Ala His Glu Ile Phe Gln
          275          280          285
Lys Ile Asp Asn Ile Leu Ile Leu Ala Cys Gly Thr Ser Tyr His Ala
          290          295          300
Gly Leu Val Gly Lys Gln Trp Ile Glu Thr Ile Ala Arg Ile Pro Val
          305          310          315          320
Asp Val His Ile Ala Ser Glu Tyr Glu Pro Thr Ile Pro Arg Ala Asn
          325          330          335
Thr Leu Val Ile Thr Ile Ser Gln Ser Gly Glu Thr Ala Asp Thr Ile
          340          345          350

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Ala Ala Leu Gln Arg Ala Gln Asn Ala Gly Met Ile Tyr Thr Leu Cys
 355 360 365

Ile Cys Asn Ser Pro Lys Ser Thr Leu Val Arg Glu Ser Val Met Lys
 370 375 380

Tyr Ile Thr Lys Cys Gly Ser Glu Val Ser Val Ala Ser Thr Lys Ala
 385 390 395 400

Phe Thr Ser Gln Leu Val Val Leu Tyr Met Leu Ala Asn Val Leu Ala
 405 410 415

Asn Lys Thr Asp Asp Leu Leu Gly Asp Leu Pro Gln Ala Ile Glu Arg
 420 425 430

Val Ile Cys Leu Thr Asn Asp Glu Met Lys His Trp Ala Asp Glu Ile
 435 440 445

Cys Asn Ala Lys Ser Ala Ile Phe Leu Gly Arg Gly Leu Asn Ala Pro
 450 455 460

Val Ala Phe Glu Gly Ala Leu Lys Leu Lys Glu Ile Ser Tyr Ile His
 465 470 475 480

Ala Glu Gly Phe Leu Gly Gly Glu Leu Lys His Gly Pro Leu Ala Leu
 485 490 495

Leu Asp Asp Lys Ile Pro Val Ile Val Thr Val Ala Asp His Ala Tyr
 500 505 510

Leu Asp His Ile Lys Ala Asn Ile Asp Glu Val Leu Ala Arg Asn Val
 515 520 525

Thr Val Tyr Ala Ile Val Asp Gln Tyr Val Asn Ile Glu Pro Gln Glu
 530 535 540

Arg Leu Arg Val Val Lys Val Pro Phe Val Ser Lys Glu Phe Ser Pro
 545 550 555 560

Ile Ile His Thr Ile Pro Met Gln Leu Leu Ser Tyr Tyr Val Ala Ile
 565 570 575

Lys Leu Gly Lys Asn Val Asp Lys Pro Arg Asn Leu Ala Lys Ser Val
 580 585 590

Thr Thr Phe
 595

<210> SEQ ID NO 7
 <211> LENGTH: 1788
 <212> TYPE: DNA
 <213> ORGANISM: Chlorella virus

<400> SEQUENCE: 7

atgtgtggca tctttggagc agtgtcaaac aacaactcta tcgaggtgtc aatcaagggt 60
 attcagaagc tagaatatcg tgggtatgat tcgtgcggtta ttgcgtatac agatgggggt 120
 gcgattgagc gtatacggtc ggttgacggc attgacgatc tgcgtaagaa aacaatcaca 180
 gaatcatcac cagtagccat tgctcactcg cgggtggagca ccaactggaat tccatcagtg 240
 gtgaacgcac atcctcatat ttctcgcgga accagtgggt gtgagtctcg tatcgcggta 300
 gtccacaacg gtatcattga aaactatcag cagatccgaa aatatctcat caatctcggc 360
 tatacgtttg atagtcaaac ggacacagag gtcattgcac atttgatcga ttctcagtac 420
 aatgggaata tcttgacacac cgtccaaatg gctgtcaagc acctgaaggc ctcttatgcc 480
 attgcagtta tgtgtcataa agagtctggt aaaatagtcg tggcgaaaca gaagtcaccc 540
 ctcgacttg gaatcggtc agatgggtgct tactacatcg cttcggacgt gctggcgctg 600
 ccgacaaata aagttgttta tatttcagat ggtttctctg cagaactatc tccagggagt 660
 atgtccattt acgatcctga tggaaatgaa gtggaatatg aagtagagga cgttgaaatg 720

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gaacaaacta gtatgtctct ctataacttt gatcattaca tgattaagga aattaatgag 780
caaccaatca gtatcctaaa cactataaaa aataaagggt tctatgcaga aatattcggg 840
gatttgggcg atgaaatctt ccaaaaaata gacaacatcc tggactggc ttgtggtaca 900
agttatcacg cgggtctcgt cgggaaacag tggatagaga ccatcgcgaa aatccccgtg 960
aatggtcata tcgcaagtga atacgaaccc accattccta aagcgaacac attggtaatc 1020
actatttcac aatcgggtga aactgaggac acgatagcgg ctttgcaacg agcccaaac 1080
gccgggatga ttacacact gtgtatttgc aattctccaa agagtactct agttcgcgag 1140
agcattatga agtacatcac taaatgtggt tctgaggtgt cagtagcatc aacgaaggcg 1200
ttacctcgc agctcgtagt actgtatata ctggcaaacg tattggcaaa taaaaccgac 1260
gatttgctgg gtgagcttcc gcaagcaata gaacgggtga ttgtttgac gagcgatgaa 1320
atgaaacaat gggctgatga aatatgcaat gcgaaatctg cgatcttcct ggggagagga 1380
ctgaacgcac cagttgcttt tgaggggtgc ttgaaactca aagagatttc ttacattcat 1440
gcgaggggct tcctgggagg tgagttgaaa cacgggtccc tcgcactcct tgatgacaag 1500
attcctgtca tcgtaactgt ggcagatcat gcttatctgg accatatcaa agcaaatatt 1560
gacgaagtgc ttgagaggaa cgtcacggta tatgccattg ttgaccagta tgtgaacatc 1620
gagccccagg aacgtcttca tatcgtcaag gttccgtttg tgtcaaaaga attttctcca 1680
ataattcaca ctatcccaat gcaactgctt tcgtattacg tggcaattaa gcttggaag 1740
aatggtgata aaccgaggaa tcttgcgaaa tctgtgacca ccttttaa 1788

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<210> SEQ ID NO 8

<211> LENGTH: 595

<212> TYPE: PRT

<213> ORGANISM: Chlorella virus

<400> SEQUENCE: 8

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Met Cys Gly Ile Phe Gly Ala Val Ser Asn Asn Asn Ser Ile Glu Val
1           5           10           15
Ser Ile Lys Gly Ile Gln Lys Leu Glu Tyr Arg Gly Tyr Asp Ser Cys
20           25           30
Gly Ile Ala Tyr Thr Asp Gly Gly Ala Ile Glu Arg Ile Arg Ser Val
35           40           45
Asp Gly Ile Asp Asp Leu Arg Lys Lys Thr Ile Thr Glu Ser Ser Pro
50           55           60
Val Ala Ile Ala His Ser Arg Trp Ser Thr Thr Gly Ile Pro Ser Val
65           70           75           80
Val Asn Ala His Pro His Ile Ser Arg Gly Thr Ser Gly Cys Glu Ser
85           90           95
Arg Ile Ala Val Val His Asn Gly Ile Ile Glu Asn Tyr Gln Gln Ile
100          105          110
Arg Lys Tyr Leu Ile Asn Leu Gly Tyr Thr Phe Asp Ser Gln Thr Asp
115          120          125
Thr Glu Val Ile Ala His Leu Ile Asp Ser Gln Tyr Asn Gly Asn Ile
130          135          140
Leu His Thr Val Gln Met Ala Val Lys His Leu Lys Gly Ser Tyr Ala
145          150          155          160
Ile Ala Val Met Cys His Lys Glu Ser Gly Lys Ile Val Val Ala Lys
165          170          175
Gln Lys Ser Pro Leu Val Leu Gly Ile Gly Ser Asp Gly Ala Tyr Tyr
180          185          190

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Ile Ala Ser Asp Val Leu Ala Leu Pro Thr Asn Lys Val Val Tyr Ile
 195 200 205
 Ser Asp Gly Phe Ser Ala Glu Leu Ser Pro Gly Ser Met Ser Ile Tyr
 210 215 220
 Asp Pro Asp Gly Asn Glu Val Glu Tyr Glu Val Glu Asp Val Glu Met
 225 230 235 240
 Glu Gln Thr Ser Met Ser Leu Tyr Asn Phe Asp His Tyr Met Ile Lys
 245 250 255
 Glu Ile Asn Glu Gln Pro Ile Ser Ile Leu Asn Thr Ile Lys Asn Lys
 260 265 270
 Gly Phe Tyr Ala Glu Ile Phe Gly Asp Leu Ala His Glu Ile Phe Gln
 275 280 285
 Lys Ile Asp Asn Ile Leu Val Leu Ala Cys Gly Thr Ser Tyr His Ala
 290 295 300
 Gly Leu Val Gly Lys Gln Trp Ile Glu Thr Ile Ala Lys Ile Pro Val
 305 310 315 320
 Asn Val His Ile Ala Ser Glu Tyr Glu Pro Thr Ile Pro Lys Ala Asn
 325 330 335
 Thr Leu Val Ile Thr Ile Ser Gln Ser Gly Glu Thr Ala Asp Thr Ile
 340 345 350
 Ala Ala Leu Gln Arg Ala Gln Asn Ala Gly Met Ile Tyr Thr Leu Cys
 355 360 365
 Ile Cys Asn Ser Pro Lys Ser Thr Leu Val Arg Glu Ser Ile Met Lys
 370 375 380
 Tyr Ile Thr Lys Cys Gly Ser Glu Val Ser Val Ala Ser Thr Lys Ala
 385 390 395 400
 Phe Thr Ser Gln Leu Val Val Leu Tyr Ile Leu Ala Asn Val Leu Ala
 405 410 415
 Asn Lys Thr Asp Asp Leu Leu Gly Glu Leu Pro Gln Ala Ile Glu Arg
 420 425 430
 Val Ile Cys Leu Thr Ser Asp Glu Met Lys Gln Trp Ala Asp Glu Ile
 435 440 445
 Cys Asn Ala Lys Ser Ala Ile Phe Leu Gly Arg Gly Leu Asn Ala Pro
 450 455 460
 Val Ala Phe Glu Gly Ala Leu Lys Leu Lys Glu Ile Ser Tyr Ile His
 465 470 475 480
 Ala Glu Gly Phe Leu Gly Gly Glu Leu Lys His Gly Pro Leu Ala Leu
 485 490 495
 Leu Asp Asp Lys Ile Pro Val Ile Val Thr Val Ala Asp His Ala Tyr
 500 505 510
 Leu Asp His Ile Lys Ala Asn Ile Asp Glu Val Leu Ala Arg Asn Val
 515 520 525
 Thr Val Tyr Ala Ile Val Asp Gln Tyr Val Asn Ile Glu Pro Gln Glu
 530 535 540
 Arg Leu His Ile Val Lys Val Pro Phe Val Ser Lys Glu Phe Ser Pro
 545 550 555 560
 Ile Ile His Thr Ile Pro Met Gln Leu Leu Ser Tyr Tyr Val Ala Ile
 565 570 575
 Lys Leu Gly Lys Asn Val Asp Lys Pro Arg Asn Leu Ala Lys Ser Val
 580 585 590
 Thr Thr Phe
 595

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<210> SEQ ID NO 9
 <211> LENGTH: 2034
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 9

atgtgtggaa tcttcgctga tctgaatddd cagcgaaca aagagagacg atacattctc 60
 gatgttctct tcaatggtct tcgctgctct gaatacagag gctacgattc tgctggaatc 120
 gccattgata attcttctcc ttcttcttct cctctcgtgt ttcgtcaagc aggaaacatt 180
 gaatcacttg ttaattccgt taacgaagag attacgaata cggatttgaa tctagacgaa 240
 gttttctact ttcattgctgg aattgcacat acgaggtggg ctactcatgg tgagccagct 300
 ccaaggaata gtcacccca atcttctggt cctggagatg attttttggg gggtcataat 360
 ggtgttatca ctaactatga ggtattgaaa gaaacgtagg tgaggcatgg atttactttt 420
 gaatcggaca cagatactga agtaattcct aagcttgcta agtttgtttt tgacaaagct 480
 aatgaagaag gtggacaaac tgttacattc tgtgaagttg tgtttgaagt gatgaggcat 540
 cttgaaggag cttatgctct tatatttaaa agctggcatt atccgaatga gtttaattgag 600
 tgcaagcttg gtagtccatt gcttttaggt gttaaagagc tagatcaagg tgagagcaat 660
 agtcatgttt tccaagatgc tcaactttcta tctaagaatg accatcccaa ggagtttttc 720
 ctatcaagtg atccacatgc tcttggtgag cacacaaaga aagttttggt gattgaagat 780
 ggcgaagttg tcaatctcaa ggatggaggt gtatcaatac ttaagtttga aaatgagagg 840
 ggaaggtgta atggtttatc gagacctgct tcagtggaac gtgccttatc tgttctagag 900
 atggaggtag agcaataaag caagggaaaa tatgatcatt acatgcaaaa ggaaatccac 960
 gagcagccag aatctttaac tactacaatg agaggccgac ttatacgcgg tggttcacgt 1020
 aaaaacgaaaa cgcctctctt aggtgggctg aaagatcacc taaagacat aagacgcagc 1080
 cggcgtatag tttttattgg atgtgggaca agttacaatg ccgctcttgc atcaagacct 1140
 atccttgaag aactctctgg tataccagtc agtatggaga ttgctagtga tctatgggac 1200
 cggcaaggtc caatatacag agaagatacc gcggtgtttg tgagtcagtc tggtgaaact 1260
 gcagatacac tacttgcttt ggactatgct cgagaaaacg gtgcattatg tgcggcata 1320
 actaacaccg ttgggagctc catagctaga aaaacacact gtggtgtcca tataaacgca 1380
 ggagctgaga ttggtgtcgc aagtacaaag gcatatacaa gtcagattgt ggtaatggta 1440
 atgctagctt tagcaatagg aagtgcacac atctccagcc aaaagagacg ggaagctata 1500
 atcgatggac tacttgattt gccgtataag gtttaaggaag tactaaagct agacgatgaa 1560
 atgaaagatc tcgcgcaact cttgatagac gagcagtcac tgctagtgtt tggcagagga 1620
 tacaactacg caacagcttt agaaggagca ttaaaagtaa aagaagtagc acttatgcac 1680
 agtgaaggaa tacttgacag agaaatgaaa catggacctt tagctttggt tgatgagaat 1740
 ctccccatag ctgtgattgc cactcgtgat gcttgtttca gtaaacaaca atctgtgatt 1800
 cagcaacttc acgcacgcaa agggagacta atagtaatgt gctcaaaagg tgatgctgca 1860
 tcggttaagct cgagtgggtc ttgtcgagct atcgaagttc ctcaagttga agattgttta 1920
 caacctgtta ttaatatagt gccattacag ttgttgctt atcatctgac tgttttgaga 1980
 ggtcacaatg ttgatcaacc gaggaatctg gcaaagagtg tgactactca atag 2034

<210> SEQ ID NO 10
 <211> LENGTH: 677
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana

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<400> SEQUENCE: 10

Met Cys Gly Ile Phe Ala Tyr Leu Asn Phe His Ala Asn Lys Glu Arg
 1 5 10 15
 Arg Tyr Ile Leu Asp Val Leu Phe Asn Gly Leu Arg Arg Leu Glu Tyr
 20 25 30
 Arg Gly Tyr Asp Ser Ala Gly Ile Ala Ile Asp Asn Ser Ser Pro Ser
 35 40 45
 Ser Ser Pro Leu Val Phe Arg Gln Ala Gly Asn Ile Glu Ser Leu Val
 50 55 60
 Asn Ser Val Asn Glu Glu Ile Thr Asn Thr Asp Leu Asn Leu Asp Glu
 65 70 75 80
 Val Phe Tyr Phe His Ala Gly Ile Ala His Thr Arg Trp Ala Thr His
 85 90 95
 Gly Glu Pro Ala Pro Arg Asn Ser His Pro Gln Ser Ser Gly Pro Gly
 100 105 110
 Asp Asp Phe Leu Val Val His Asn Gly Val Ile Thr Asn Tyr Glu Val
 115 120 125
 Leu Lys Glu Thr Leu Val Arg His Gly Phe Thr Phe Glu Ser Asp Thr
 130 135 140
 Asp Thr Glu Val Ile Pro Lys Leu Ala Lys Phe Val Phe Asp Lys Ala
 145 150 155 160
 Asn Glu Glu Gly Gly Gln Thr Val Thr Phe Cys Glu Val Val Phe Glu
 165 170 175
 Val Met Arg His Leu Glu Gly Ala Tyr Ala Leu Ile Phe Lys Ser Trp
 180 185 190
 His Tyr Pro Asn Glu Leu Ile Ala Cys Lys Leu Gly Ser Pro Leu Leu
 195 200 205
 Leu Gly Val Lys Glu Leu Asp Gln Gly Glu Ser Asn Ser His Val Phe
 210 215 220
 Gln Asp Ala His Phe Leu Ser Lys Asn Asp His Pro Lys Glu Phe Phe
 225 230 235 240
 Leu Ser Ser Asp Pro His Ala Leu Val Glu His Thr Lys Lys Val Leu
 245 250 255
 Val Ile Glu Asp Gly Glu Val Val Asn Leu Lys Asp Gly Gly Val Ser
 260 265 270
 Ile Leu Lys Phe Glu Asn Glu Arg Gly Arg Cys Asn Gly Leu Ser Arg
 275 280 285
 Pro Ala Ser Val Glu Arg Ala Leu Ser Val Leu Glu Met Glu Val Glu
 290 295 300
 Gln Ile Ser Lys Gly Lys Tyr Asp His Tyr Met Gln Lys Glu Ile His
 305 310 315 320
 Glu Gln Pro Glu Ser Leu Thr Thr Thr Met Arg Gly Arg Leu Ile Arg
 325 330 335
 Gly Gly Ser Arg Lys Thr Lys Thr Val Leu Leu Gly Gly Leu Lys Asp
 340 345 350
 His Leu Lys Thr Ile Arg Arg Ser Arg Arg Ile Val Phe Ile Gly Cys
 355 360 365
 Gly Thr Ser Tyr Asn Ala Ala Leu Ala Ser Arg Pro Ile Leu Glu Glu
 370 375 380
 Leu Ser Gly Ile Pro Val Ser Met Glu Ile Ala Ser Asp Leu Trp Asp
 385 390 395 400
 Arg Gln Gly Pro Ile Tyr Arg Glu Asp Thr Ala Val Phe Val Ser Gln
 405 410 415

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Ser Gly Glu Thr Ala Asp Thr Leu Leu Ala Leu Asp Tyr Ala Arg Glu
420 425 430

Asn Gly Ala Leu Cys Val Gly Ile Thr Asn Thr Val Gly Ser Ser Ile
435 440 445

Ala Arg Lys Thr His Cys Gly Val His Ile Asn Ala Gly Ala Glu Ile
450 455 460

Gly Val Ala Ser Thr Lys Ala Tyr Thr Ser Gln Ile Val Val Met Val
465 470 475 480

Met Leu Ala Leu Ala Ile Gly Ser Asp Thr Ile Ser Ser Gln Lys Arg
485 490 495

Arg Glu Ala Ile Ile Asp Gly Leu Leu Asp Leu Pro Tyr Lys Val Lys
500 505 510

Glu Val Leu Lys Leu Asp Asp Glu Met Lys Asp Leu Ala Gln Leu Leu
515 520 525

Ile Asp Glu Gln Ser Leu Leu Val Phe Gly Arg Gly Tyr Asn Tyr Ala
530 535 540

Thr Ala Leu Glu Gly Ala Leu Lys Val Lys Glu Val Ala Leu Met His
545 550 555 560

Ser Glu Gly Ile Leu Ala Gly Glu Met Lys His Gly Pro Leu Ala Leu
565 570 575

Val Asp Glu Asn Leu Pro Ile Ala Val Ile Ala Thr Arg Asp Ala Cys
580 585 590

Phe Ser Lys Gln Gln Ser Val Ile Gln Gln Leu His Ala Arg Lys Gly
595 600 605

Arg Leu Ile Val Met Cys Ser Lys Gly Asp Ala Ala Ser Val Ser Ser
610 615 620

Ser Gly Ser Cys Arg Ala Ile Glu Val Pro Gln Val Glu Asp Cys Leu
625 630 635 640

Gln Pro Val Ile Asn Ile Val Pro Leu Gln Leu Leu Ala Tyr His Leu
645 650 655

Thr Val Leu Arg Gly His Asn Val Asp Gln Pro Arg Asn Leu Ala Lys
660 665 670

Ser Val Thr Thr Gln
675

<210> SEQ ID NO 11
<211> LENGTH: 1170
<212> TYPE: DNA
<213> ORGANISM: Chlorella virus

<400> SEQUENCE: 11

atgtcacgaa tcgcagtcgt tggttgtggt tacgtcggaa ccgcttgtgc agtacttctt 60
gctcaaaaaa acgaagtcac cgtgcttgat attagcgaag accgtgttca gctaatacaag 120
aacaagaaga gtccaatcga ggacaaggaa atcgaagagt ttctcgaaac gaaagacctg 180
aacctgaccg cgacgactga caaggttctt gcatacgaaa acgccgaatt tgtcatcatc 240
gcaacccccga ctgactatga cgtgggttact aggtatttta acacgaaatc tgtggaaagc 300
gttatcgggg atgtgatcaa aaatacacgg acccagccaa ctattgtgat taaatctacc 360
atccccattg gatttgttga taaggttcgt gagcaattca actacagcaa cattatattc 420
tctccggagt ttctgcgaga aggtagggca ttgtatgata atctctatcc atcgcgtatt 480
atcgtaggag atgattcccc cattgcgctt aagttcgcaa accttctcgt tgaaggttct 540
aaaaccccac ttgcacctgt cctgacgatg gggactcgtg aagccgagc cgtcaaaacta 600

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ttctctaaca cgtatcttgc gatgaggtt gcatatttca acgaactaga tacgtttgca 660
ttgtctcatg gtatgagtgc gaaagaaatc attgacggtg tgactctgga gcctcgaatt 720
ggtcagggtt actcaaacc ttcgttcggt tacggagctt attgcttccc aaaggatagc 780
aagcaacttc tggctaactt tgagggggtg cctcaaaata tcatcggggc aattgtagaa 840
tcaaatgaaa ctcgcaagga agcgattgta agtgaagtag aaaatcgttt tcccacgact 900
gttggtgtgt ataagctcgc tgctaaagcg ggttctgata attttaggag ttctgcaatt 960
gtagacataa tggagcgcact tgcaaacagg ggttatcaca ttaagatttt cgaaccaact 1020
gtggaacaat tcgaaaactt tgaagttgat aacaacctga caacatttgc gactgagagc 1080
gatgtaatta tcgcaaacag agttcccggt gaacatcgca ttctctttgg taaaaaattg 1140
atcacacgtg atgtatatgg cgataactaa 1170

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<210> SEQ ID NO 12

<211> LENGTH: 389

<212> TYPE: PRT

<213> ORGANISM: Chlorella virus

<400> SEQUENCE: 12

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Met Ser Arg Ile Ala Val Val Gly Cys Gly Tyr Val Gly Thr Ala Cys
1           5           10           15

Ala Val Leu Leu Ala Gln Lys Asn Glu Val Thr Val Leu Asp Ile Ser
20           25           30

Glu Asp Arg Val Gln Leu Ile Lys Asn Lys Lys Ser Pro Ile Glu Asp
35           40           45

Lys Glu Ile Glu Glu Phe Leu Glu Thr Lys Asp Leu Asn Leu Thr Ala
50           55           60

Thr Thr Asp Lys Val Leu Ala Tyr Glu Asn Ala Glu Phe Val Ile Ile
65           70           75           80

Ala Thr Pro Thr Asp Tyr Asp Val Val Thr Arg Tyr Phe Asn Thr Lys
85           90           95

Ser Val Glu Ser Val Ile Gly Asp Val Ile Lys Asn Thr Arg Thr Gln
100          105          110

Pro Thr Ile Val Ile Lys Ser Thr Ile Pro Ile Gly Phe Val Asp Lys
115          120          125

Val Arg Glu Gln Phe Asn Tyr Ser Asn Ile Ile Phe Ser Pro Glu Phe
130          135          140

Leu Arg Glu Gly Arg Ala Leu Tyr Asp Asn Leu Tyr Pro Ser Arg Ile
145          150          155          160

Ile Val Gly Asp Asp Ser Pro Ile Ala Leu Lys Phe Ala Asn Leu Leu
165          170          175

Val Glu Gly Ser Lys Thr Pro Leu Ala Pro Val Leu Thr Met Gly Thr
180          185          190

Arg Glu Ala Glu Ala Val Lys Leu Phe Ser Asn Thr Tyr Leu Ala Met
195          200          205

Arg Val Ala Tyr Phe Asn Glu Leu Asp Thr Phe Ala Leu Ser His Gly
210          215          220

Met Ser Ala Lys Glu Ile Ile Asp Gly Val Thr Leu Glu Pro Arg Ile
225          230          235          240

Gly Gln Gly Tyr Ser Asn Pro Ser Phe Gly Tyr Gly Ala Tyr Cys Phe
245          250          255

Pro Lys Asp Thr Lys Gln Leu Leu Ala Asn Phe Glu Gly Val Pro Gln
260          265          270

Asn Ile Ile Gly Ala Ile Val Glu Ser Asn Glu Thr Arg Lys Glu Ala

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275	280	285
Ile Val Ser Glu Val Glu Asn Arg Phe Pro Thr Thr Val Gly Val Tyr 290	295	300
Lys Leu Ala Ala Lys Ala Gly Ser Asp Asn Phe Arg Ser Ser Ala Ile 305	310	315 320
Val Asp Ile Met Glu Arg Leu Ala Asn Arg Gly Tyr His Ile Lys Ile 325	330	335
Phe Glu Pro Thr Val Glu Gln Phe Glu Asn Phe Glu Val Asp Asn Asn 340	345	350
Leu Thr Thr Phe Ala Thr Glu Ser Asp Val Ile Ile Ala Asn Arg Val 355	360	365
Pro Val Glu His Arg Ile Leu Phe Gly Lys Lys Leu Ile Thr Arg Asp 370	375	380
Val Tyr Gly Asp Asn 385		

<210> SEQ ID NO 13
 <211> LENGTH: 1170
 <212> TYPE: DNA
 <213> ORGANISM: Chlorella virus

<400> SEQUENCE: 13

```

atgtcacgaa tcgcagtcgt tggttgtggt tacgtcggaa ccgcttgtgc agtacttctt    60
gctcaaaaaa acgaagtcac cgtgcttgat attagtgaag accgtgttca gctaatcaag    120
aacaagaaga gtccaatcga ggacaaggaa atcgaagagt ttctcgaaac gaaagacctg    180
aacctgaccg cgacgactga caaggttctt gcatacgaaa acgccgaatt tgtcatcatc    240
gcaaccccca ctgactatga cgtgggttact aggtatttta acacgaaatc tgtggaaagc    300
gttatcgggg atgtgatcga aaatacacgg acccagccaa ctattgtgat taaatctacc    360
atccccattg gatttgttga taaggttcgt gagcaattca actacagcaa cattatattc    420
tctccggagt ttctgcgcga aggtagggca ttgtatgata atctctatcc atcgcgtatt    480
atcgtaggag atgattcccc cattgcgctt aagttcgcaa accttctcgt tgaaggttct    540
aaaactccgc ttgccctgt cctgacgatg ggaactcgcg aagccgaggc cgtcaaaacta    600
ttctctaaca cgtatcttgc aatgcgagtt gcatacttca acgaactaga tacattcgca    660
atgtctcatg gtatgaatgc gaaagaaatc attgacggtg tgactttgga gcctcgcatt    720
ggtcaggggg actcaaacc ttcgttcggg tatggagctt attgctttcc gaaggatagc    780
aagcaactgc tggctaattt cgaggggggtg cctcaagata taatcggggc aattgtagaa    840
tcaaatgaaa ctcgcaagga agcgattgta agtgaagtag aaaatcgttt tcccacgact    900
gttgggtgtg ataagctcgc tgctaaagcg ggttctgata attttagaag ttctgcaatt    960
gtagacataa tggagcgact tgcaaacagg ggttatcaca ttaagatttt cgaaccaact   1020
gtggaacaat tcgaaaactt tgaagttgat aacaacctga caacatttgc gactgatagc   1080
gatgtaatta tcgcaaacag agttcccgtt gaacatcgca ttctctttgg taaaaaattg   1140
atcacacgtg atgtatatgg cgataactaa                                     1170

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<210> SEQ ID NO 14
 <211> LENGTH: 389
 <212> TYPE: PRT
 <213> ORGANISM: Chlorella virus

<400> SEQUENCE: 14

Met Ser Arg Ile Ala Val Val Gly Cys Gly Tyr Val Gly Thr Ala Cys

-continued

1	5	10	15
Ala Val Leu Leu Ala Gln Lys Asn Glu Val Thr Val Leu Asp Ile Ser	20	25	30
Glu Asp Arg Val Gln Leu Ile Lys Asn Lys Lys Ser Pro Ile Glu Asp	35	40	45
Lys Glu Ile Glu Glu Phe Leu Glu Thr Lys Asp Leu Asn Leu Thr Ala	50	55	60
Thr Thr Asp Lys Val Leu Ala Tyr Glu Asn Ala Glu Phe Val Ile Ile	65	70	75
Ala Thr Pro Thr Asp Tyr Asp Val Val Thr Arg Tyr Phe Asn Thr Lys	85	90	95
Ser Val Glu Ser Val Ile Gly Asp Val Ile Glu Asn Thr Arg Thr Gln	100	105	110
Pro Thr Ile Val Ile Lys Ser Thr Ile Pro Ile Gly Phe Val Asp Lys	115	120	125
Val Arg Glu Gln Phe Asn Tyr Ser Asn Ile Ile Phe Ser Pro Glu Phe	130	135	140
Leu Arg Glu Gly Arg Ala Leu Tyr Asp Asn Leu Tyr Pro Ser Arg Ile	145	150	155
Ile Val Gly Asp Asp Ser Pro Ile Ala Leu Lys Phe Ala Asn Leu Leu	165	170	175
Val Glu Gly Ser Lys Thr Pro Leu Ala Pro Val Leu Thr Met Gly Thr	180	185	190
Arg Glu Ala Glu Ala Val Lys Leu Phe Ser Asn Thr Tyr Leu Ala Met	195	200	205
Arg Val Ala Tyr Phe Asn Glu Leu Asp Thr Phe Ala Met Ser His Gly	210	215	220
Met Asn Ala Lys Glu Ile Ile Asp Gly Val Thr Leu Glu Pro Arg Ile	225	230	235
Gly Gln Gly Tyr Ser Asn Pro Ser Phe Gly Tyr Gly Ala Tyr Cys Phe	245	250	255
Pro Lys Asp Thr Lys Gln Leu Leu Ala Asn Phe Glu Gly Val Pro Gln	260	265	270
Asp Ile Ile Gly Ala Ile Val Glu Ser Asn Glu Thr Arg Lys Glu Ala	275	280	285
Ile Val Ser Glu Val Glu Asn Arg Phe Pro Thr Thr Val Gly Val Tyr	290	295	300
Lys Leu Ala Ala Lys Ala Gly Ser Asp Asn Phe Arg Ser Ser Ala Ile	305	310	315
Val Asp Ile Met Glu Arg Leu Ala Asn Arg Gly Tyr His Ile Lys Ile	325	330	335
Phe Glu Pro Thr Val Glu Gln Phe Glu Asn Phe Glu Val Asp Asn Asn	340	345	350
Leu Thr Thr Phe Ala Thr Asp Ser Asp Val Ile Ile Ala Asn Arg Val	355	360	365
Pro Val Glu His Arg Ile Leu Phe Gly Lys Lys Leu Ile Thr Arg Asp	370	375	380
Val Tyr Gly Asp Asn	385		

<210> SEQ ID NO 15

<211> LENGTH: 1443

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

-continued

<400> SEQUENCE: 15

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atggtgaaga tctgttgtat tggagctgga tatgtaggag gaccaacaat ggcagtgatt    60
gcattgaaat gtccagatat tgaagtggca gttgttgata tctctgttcc tagaatcaac    120
gcttggaaaca gtgatcagct tccgatttac gagccaggtc ttgacgatat cgtaagcaa    180
tgcagaggaa agaatctttt cttcagtact gatgtggaga aacatgttag agaagctgat    240
attgtctttg tctctgttaa cacaccgact aaaacgactg gtcttggagc tgggaaagct    300
gctgatctca cttattggga gagtgctgct cgtatgatcg cggatgtatc ggtttctgac    360
aagattggtg ttgagaaatc gactgttccg gtgaagacag ctgaagctat tgagaagatt    420
ttgatgcata acagtaaagg aatcaagttt cagattcttt cgaatccgga gtttcttgct    480
gaaggaactg ctatcgctga tctttttaac cctgaccgtg ttttgatcgg agggcgagaa    540
acacctgaag gattcaaagc tgttcagaca cttaaagagg tttatgctaa ttgggttctc    600
gaaggtcaga tcatcacaac taatctctgg tctgctgagc tttctaagtt agctgcaaat    660
gctttcttgg ctacagaggat ttcacagtc aatgccatgt ctgcactttg tgaatccact    720
ggtgctgatg ttactcaagt gtcttacgct gttggtactg attcaagaat cggttccaaa    780
ttcttgaacg ctagtgttgg attcggaggt tcttgtttcc agaaggacat tctgaatctc    840
gtctacatct gtcaatgcaa cggacttcca gaagtggcgg aatactggaa acaagtgatc    900
aagatcaacg attaccaaaa gaaccggttc gtgaacagaa tcgtgtctc tatgttcaac    960
actgtctcca acaagaaggt tgcgattctt ggattcgcac tcaagaaaga cactggtgac   1020
acaagggaaa cacctgccat tgatgtgtgt aaaggtctat taggagacaa agcacagatc   1080
agtatctatg atcctcaagt cacagaggaa cagattcaga gagatctctc gatgaaaaag   1140
ttcgactggg accatcctct tcaactgcag ccaatgagtc caaccacagt gaaacaagtg   1200
agtgtgactt gggacgcata tgaagctaca aaagacgcac acgcggtttg cgttttgact   1260
gagtgggacg agtttaagtc gttagattac cagaagatct tcgacaacat gcagaaaccg   1320
gcttttatct tcgacggaag aacattatg aatgttaaca agttaagaga gattggtttc   1380
attgtttact ccattggtaa gccacttgac ccatggctca aggacatgcc tgcctttgtc   1440
taa                                                                    1443

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<210> SEQ ID NO 16

<211> LENGTH: 480

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 16

```

Met Val Lys Ile Cys Cys Ile Gly Ala Gly Tyr Val Gly Gly Pro Thr
1           5           10           15
Met Ala Val Ile Ala Leu Lys Cys Pro Asp Ile Glu Val Ala Val Val
          20           25           30
Asp Ile Ser Val Pro Arg Ile Asn Ala Trp Asn Ser Asp Gln Leu Pro
          35           40           45
Ile Tyr Glu Pro Gly Leu Asp Asp Ile Val Lys Gln Cys Arg Gly Lys
          50           55           60
Asn Leu Phe Phe Ser Thr Asp Val Glu Lys His Val Arg Glu Ala Asp
          65           70           75           80
Ile Val Phe Val Ser Val Asn Thr Pro Thr Lys Thr Thr Gly Leu Gly
          85           90           95
Ala Gly Lys Ala Ala Asp Leu Thr Tyr Trp Glu Ser Ala Ala Arg Met
          100           105           110

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Ile Ala Asp Val Ser Val Ser Asp Lys Ile Val Val Glu Lys Ser Thr
 115 120 125
 Val Pro Val Lys Thr Ala Glu Ala Ile Glu Lys Ile Leu Met His Asn
 130 135 140
 Ser Lys Gly Ile Lys Phe Gln Ile Leu Ser Asn Pro Glu Phe Leu Ala
 145 150 155 160
 Glu Gly Thr Ala Ile Ala Asp Leu Phe Asn Pro Asp Arg Val Leu Ile
 165 170 175
 Gly Gly Arg Glu Thr Pro Glu Gly Phe Lys Ala Val Gln Thr Leu Lys
 180 185 190
 Glu Val Tyr Ala Asn Trp Val Pro Glu Gly Gln Ile Ile Thr Thr Asn
 195 200 205
 Leu Trp Ser Ala Glu Leu Ser Lys Leu Ala Ala Asn Ala Phe Leu Ala
 210 215 220
 Gln Arg Ile Ser Ser Val Asn Ala Met Ser Ala Leu Cys Glu Ser Thr
 225 230 235 240
 Gly Ala Asp Val Thr Gln Val Ser Tyr Ala Val Gly Thr Asp Ser Arg
 245 250 255
 Ile Gly Ser Lys Phe Leu Asn Ala Ser Val Gly Phe Gly Gly Ser Cys
 260 265 270
 Phe Gln Lys Asp Ile Leu Asn Leu Val Tyr Ile Cys Gln Cys Asn Gly
 275 280 285
 Leu Pro Glu Val Ala Glu Tyr Trp Lys Gln Val Ile Lys Ile Asn Asp
 290 295 300
 Tyr Gln Lys Asn Arg Phe Val Asn Arg Ile Val Ser Ser Met Phe Asn
 305 310 315 320
 Thr Val Ser Asn Lys Lys Val Ala Ile Leu Gly Phe Ala Phe Lys Lys
 325 330 335
 Asp Thr Gly Asp Thr Arg Glu Thr Pro Ala Ile Asp Val Cys Lys Gly
 340 345 350
 Leu Leu Gly Asp Lys Ala Gln Ile Ser Ile Tyr Asp Pro Gln Val Thr
 355 360 365
 Glu Glu Gln Ile Gln Arg Asp Leu Ser Met Lys Lys Phe Asp Trp Asp
 370 375 380
 His Pro Leu His Leu Gln Pro Met Ser Pro Thr Thr Val Lys Gln Val
 385 390 395 400
 Ser Val Thr Trp Asp Ala Tyr Glu Ala Thr Lys Asp Ala His Ala Val
 405 410 415
 Cys Val Leu Thr Glu Trp Asp Glu Phe Lys Ser Leu Asp Tyr Gln Lys
 420 425 430
 Ile Phe Asp Asn Met Gln Lys Pro Ala Phe Ile Phe Asp Gly Arg Asn
 435 440 445
 Ile Met Asn Val Asn Lys Leu Arg Glu Ile Gly Phe Ile Val Tyr Ser
 450 455 460
 Ile Gly Lys Pro Leu Asp Pro Trp Leu Lys Asp Met Pro Ala Phe Val
 465 470 475 480

<210> SEQ ID NO 17
 <211> LENGTH: 1443
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 17

atggtgaaga tatgttgat tggagctggg tatgttggtg gaccaacaat ggcagtgatt 60

-continued

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gcattgaaat gtccagacgt tgaagtagcg gttgttgata tctctgtacc acgtatcaac 120
gcttggaaaca gtgacacgct tccgatttac gagcctggtc ttgatgatgt tgtgaagcaa 180
tgccgtggca agaacctttt ctttagtact gatgttgaga aacatgtag ggaagctgat 240
attgtgtttg tttctgtcaa cacaccgact aagactagag gtcttgggtc tggtaaagct 300
gcggatctta cgtactggga gagcgctgcg cgtatgatcg ctgatgtttc ggtatcggat 360
aagattgtcg ttgagaaatc gactgttccg gttaaaacag ctgaagctat tgagaagatt 420
ttgacacata acagtaaagg gattaagttt cagattcttt cgaatcccga gtttttggcg 480
gaaggaaccg cgattaagga cctatttaat ccggaccgtg ttcttatcgg agggcgggaa 540
accccagaag ggtttaaagc ggtgcagact ctcaagaatg tgtatgcaca ctgggttctt 600
gaaggccaaa tcataacaac caatctctgg tctgctgagc tgtccaagct tgccggcaaac 660
gctttcttgg ctcaaaggat ttcactcagt aatgctatgt cggctctgtg tgaagccaca 720
ggcgcagatg tcacgcaagt gtcttacgcg gttggtacag actcaaggat tgggtccaag 780
ttcttgaact cgagtgttgg attcgggtgg tctgttttcc agaaggacat tctgaatctt 840
gtctacatct gtgagtgcaa cggactcccg gaagtggcag agtactggaa gcaagtcatc 900
aagatcaatg actaccagaa gagccgggtc gtgaaccgtg ttgtttcttc catgttcaac 960
tctgtatcaa acaagaagat tgccggttctc ggtttcgcac tcaagaaaga caccggtgac 1020
acaagggaga ctccagccat cgatgtgtgc aagggtcttt tagaagaca agcaaggcta 1080
agcatttacg acccacaagt gactgaggat cagatccaga gggatttatc catgaacaag 1140
ttcgactggg accatctct acatttgcag ccaatgagcc caacaacagt gaaacaagtg 1200
accgttactt gggacgcata cgaagcaact aaggacgctc acggtatctg catcatgacc 1260
gagtgggatg agttcaagaa ccttgatttc cagaagatct ttgacaacat gcagaaacca 1320
gctttcgtgt tcgatggaag aacattatg aatctgcaaa agctaaggga gattggtttc 1380
attgtttact ccattggtaa gcctctcgac gactggctca aggacatgcc tgccgttgcc 1440
taa 1443

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<210> SEQ ID NO 18

<211> LENGTH: 480

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 18

```

Met Val Lys Ile Cys Cys Ile Gly Ala Gly Tyr Val Gly Gly Pro Thr
1           5           10           15

Met Ala Val Ile Ala Leu Lys Cys Pro Asp Val Glu Val Ala Val Val
          20           25           30

Asp Ile Ser Val Pro Arg Ile Asn Ala Trp Asn Ser Asp Thr Leu Pro
          35           40           45

Ile Tyr Glu Pro Gly Leu Asp Asp Val Val Lys Gln Cys Arg Gly Lys
          50           55           60

Asn Leu Phe Phe Ser Thr Asp Val Glu Lys His Val Arg Glu Ala Asp
65           70           75           80

Ile Val Phe Val Ser Val Asn Thr Pro Thr Lys Thr Arg Gly Leu Gly
          85           90           95

Ala Gly Lys Ala Ala Asp Leu Thr Tyr Trp Glu Ser Ala Ala Arg Met
          100          105          110

Ile Ala Asp Val Ser Val Ser Asp Lys Ile Val Val Glu Lys Ser Thr
          115          120          125

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Val	Pro	Val	Lys	Thr	Ala	Glu	Ala	Ile	Glu	Lys	Ile	Leu	Thr	His	Asn					
	130					135					140									
Ser	Lys	Gly	Ile	Lys	Phe	Gln	Ile	Leu	Ser	Asn	Pro	Glu	Phe	Leu	Ala					
145					150					155					160					
Glu	Gly	Thr	Ala	Ile	Lys	Asp	Leu	Phe	Asn	Pro	Asp	Arg	Val	Leu	Ile					
				165					170					175						
Gly	Gly	Arg	Glu	Thr	Pro	Glu	Gly	Phe	Lys	Ala	Val	Gln	Thr	Leu	Lys					
			180					185					190							
Asn	Val	Tyr	Ala	His	Trp	Val	Pro	Glu	Gly	Gln	Ile	Ile	Thr	Thr	Asn					
	195					200						205								
Leu	Trp	Ser	Ala	Glu	Leu	Ser	Lys	Leu	Ala	Ala	Asn	Ala	Phe	Leu	Ala					
210						215					220									
Gln	Arg	Ile	Ser	Ser	Val	Asn	Ala	Met	Ser	Ala	Leu	Cys	Glu	Ala	Thr					
225					230					235					240					
Gly	Ala	Asp	Val	Thr	Gln	Val	Ser	Tyr	Ala	Val	Gly	Thr	Asp	Ser	Arg					
				245					250					255						
Ile	Gly	Pro	Lys	Phe	Leu	Asn	Ser	Ser	Val	Gly	Phe	Gly	Gly	Ser	Cys					
			260					265					270							
Phe	Gln	Lys	Asp	Ile	Leu	Asn	Leu	Val	Tyr	Ile	Cys	Glu	Cys	Asn	Gly					
		275					280					285								
Leu	Pro	Glu	Val	Ala	Glu	Tyr	Trp	Lys	Gln	Val	Ile	Lys	Ile	Asn	Asp					
290						295					300									
Tyr	Gln	Lys	Ser	Arg	Phe	Val	Asn	Arg	Val	Val	Ser	Ser	Met	Phe	Asn					
305					310					315					320					
Ser	Val	Ser	Asn	Lys	Lys	Ile	Ala	Val	Leu	Gly	Phe	Ala	Phe	Lys	Lys					
				325					330					335						
Asp	Thr	Gly	Asp	Thr	Arg	Glu	Thr	Pro	Ala	Ile	Asp	Val	Cys	Lys	Gly					
			340					345					350							
Leu	Leu	Glu	Asp	Lys	Ala	Arg	Leu	Ser	Ile	Tyr	Asp	Pro	Gln	Val	Thr					
		355					360					365								
Glu	Asp	Gln	Ile	Gln	Arg	Asp	Leu	Ser	Met	Asn	Lys	Phe	Asp	Trp	Asp					
370						375					380									
His	Pro	Leu	His	Leu	Gln	Pro	Met	Ser	Pro	Thr	Thr	Val	Lys	Gln	Val					
385					390					395					400					
Thr	Val	Thr	Trp	Asp	Ala	Tyr	Glu	Ala	Thr	Lys	Asp	Ala	His	Gly	Ile					
				405					410					415						
Cys	Ile	Met	Thr	Glu	Trp	Asp	Glu	Phe	Lys	Asn	Leu	Asp	Phe	Gln	Lys					
			420					425					430							
Ile	Phe	Asp	Asn	Met	Gln	Lys	Pro	Ala	Phe	Val	Phe	Asp	Gly	Arg	Asn					
		435					440					445								
Ile	Met	Asn	Leu	Gln	Lys	Leu	Arg	Glu	Ile	Gly	Phe	Ile	Val	Tyr	Ser					
450						455					460									
Ile	Gly	Lys	Pro	Leu	Asp	Asp	Trp	Leu	Lys	Asp	Met	Pro	Ala	Val	Ala					
465					470					475					480					

<210> SEQ ID NO 19

<211> LENGTH: 1443

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 19

atggtgaaga tttgctgcat tggagctgga tatggtggtg gtccaacat ggctgtcatt 60

gctctaaagt gtccatctgt tgaagtagct gttggtgata tctctgtgcc aaggatcaat 120

gcctggaaca gtgatcagtt accgatctat gagcctggtc ttgatgatgt cgtaagcag 180

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tgccgtggaa agaatctctt cttcagcacc gatgttgaga aacatgtgag agaggctgac 240
attgtttttg tgtctgtcaa caccctact aagaccctg gtcttgagc tggcaaagct 300
gcgatttga cttactggga gagcgctgct cgtatgattg ccgatgttc ggtttccgac 360
aagattggtg ttgagaaatc aactgttctt gtcaaaaaccg cagaggcaat tgagaagatt 420
cttacacaca acagcaaagg aatcaaattc cagattctgt caaacctga gttccttgct 480
gaaggaaccg ctattgaaga ctttttcatg cctgaccgtg tctcatcgg tggtcgtgaa 540
acaactgaag gctttgcagc cgtcaaagcc ttgaaagaca tttatgccca atgggtccct 600
gaagagagaa tctcaccac caatctatgg tctgccgagc tttccaagct tgcagctaat 660
gccttctag cccagagaat ctcatcagtc aatgcaatgt ccgctctctg tgaggcaact 720
ggcgccaatg tctcagaggt ctcttatgct gtgggcaaag actctcgtat tggteccaag 780
ttcttgaact ctagtgttg gttcggagga tcttgtttcc agaaagatat tctcaactta 840
gtctacatct gcgaatgcaa cggcttacc gaagttgctg agtactggaa acaagtcac 900
aagatcaacg actaccagaa aaccgattt gttaaccgca ttgtctctc aatgtttaac 960
acagtctcca acaaaaagat tgcggttctc ggcttcgctt tcaagaaaga cactggagac 1020
actagagaga ctccagccat tgatgtctgc aaaggtctgt taggtgacaa ggctcgtctc 1080
agcatctacg acccacaagt cactgaagag cagatccaaa gagacttaac catgaacaaa 1140
ttcgactggg accaccact tcatctccag cccatgagcc ccaccactgt gaagcaagtc 1200
tcagtcgctt gggacgcata cactgcaacc aaagacgcc acggtatctg cattttaacc 1260
gagtgggacg agttcaagaa acttgatttc cagcggatct ttgagaatat gcagaaaccg 1320
gcttttgttt ttgacggtag aaacgtggtc gacgctgata aactcagga gattgggttt 1380
attgtttact ccattggtaa gccattggac cagtggctca aggacatgcc tgctcttgcc 1440
taa 1443

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<210> SEQ ID NO 20

<211> LENGTH: 480

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 20

```

Met Val Lys Ile Cys Cys Ile Gly Ala Gly Tyr Val Gly Gly Pro Thr
1          5          10          15
Met Ala Val Ile Ala Leu Lys Cys Pro Ser Val Glu Val Ala Val Val
20          25          30
Asp Ile Ser Val Pro Arg Ile Asn Ala Trp Asn Ser Asp Gln Leu Pro
35          40          45
Ile Tyr Glu Pro Gly Leu Asp Asp Val Val Lys Gln Cys Arg Gly Lys
50          55          60
Asn Leu Phe Phe Ser Thr Asp Val Glu Lys His Val Arg Glu Ala Asp
65          70          75          80
Ile Val Phe Val Ser Val Asn Thr Pro Thr Lys Thr Arg Gly Leu Gly
85          90          95
Ala Gly Lys Ala Ala Asp Leu Thr Tyr Trp Glu Ser Ala Ala Arg Met
100         105         110
Ile Ala Asp Val Ser Val Ser Asp Lys Ile Val Val Glu Lys Ser Thr
115         120         125
Val Pro Val Lys Thr Ala Glu Ala Ile Glu Lys Ile Leu Thr His Asn
130         135         140
Ser Lys Gly Ile Lys Phe Gln Ile Leu Ser Asn Pro Glu Phe Leu Ala

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145		150		155		160
Glu Gly Thr Ala Ile	Glu Asp Leu Phe Met Pro Asp Arg Val Leu Ile					
	165		170		175	
Gly Gly Arg Glu Thr Thr	Glu Gly Phe Ala Ala Val Lys Ala Leu Lys					
	180		185		190	
Asp Ile Tyr Ala Gln Trp Val	Pro Glu Glu Arg Ile Leu Thr Thr Asn					
	195		200		205	
Leu Trp Ser Ala Glu Leu Ser	Lys Leu Ala Ala Asn Ala Phe Leu Ala					
	210		215		220	
Gln Arg Ile Ser Ser Val	Asn Ala Met Ser Ala Leu Cys Glu Ala Thr					
	225		230		235	240
Gly Ala Asn Val Ser Glu Val	Ser Tyr Ala Val Gly Lys Asp Ser Arg					
	245		250		255	
Ile Gly Pro Lys Phe Leu Asn	Ser Ser Val Gly Phe Gly Gly Ser Cys					
	260		265		270	
Phe Gln Lys Asp Ile Leu Asn	Leu Val Tyr Ile Cys Glu Cys Asn Gly					
	275		280		285	
Leu Pro Glu Val Ala Glu Tyr	Trp Lys Gln Val Ile Lys Ile Asn Asp					
	290		295		300	
Tyr Gln Lys Thr Arg Phe Val	Asn Arg Ile Val Ser Ser Met Phe Asn					
	305		310		315	320
Thr Val Ser Asn Lys Lys Ile	Ala Val Leu Gly Phe Ala Phe Lys Lys					
	325		330		335	
Asp Thr Gly Asp Thr Arg Glu	Thr Pro Ala Ile Asp Val Cys Lys Gly					
	340		345		350	
Leu Leu Gly Asp Lys Ala Arg	Leu Ser Ile Tyr Asp Pro Gln Val Thr					
	355		360		365	
Glu Glu Gln Ile Gln Arg Asp	Leu Thr Met Asn Lys Phe Asp Trp Asp					
	370		375		380	
His Pro Leu His Leu Gln Pro	Met Ser Pro Thr Thr Val Lys Gln Val					
	385		390		395	400
Ser Val Ala Trp Asp Ala Tyr	Thr Ala Thr Lys Asp Ala His Gly Ile					
	405		410		415	
Cys Ile Leu Thr Glu Trp Asp	Glu Phe Lys Lys Leu Asp Phe Gln Arg					
	420		425		430	
Ile Phe Glu Asn Met Gln Lys	Pro Ala Phe Val Phe Asp Gly Arg Asn					
	435		440		445	
Val Val Asp Ala Asp Lys Leu	Arg Glu Ile Gly Phe Ile Val Tyr Ser					
	450		455		460	
Ile Gly Lys Pro Leu Asp Gln	Trp Leu Lys Asp Met Pro Ala Leu Ala					
	465		470		475	480

<210> SEQ ID NO 21

<211> LENGTH: 1446

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 21

atggtgaaga tatgctgcat aggagctggt tatgtgggtg gtccaacat ggcggtgatg	60
gctcttaagt gtctgagat tgaagtagtc gttgtggata tctctgaacc aaggatcaat	120
gcttgaaca gtgataggct tcctatttac gagccggat tggaagatgt ggtgaaacaa	180
tgcagaggga aaaacctctt ctttagcaca gacgtggaga aacatgtatt tgagagtgat	240
attgtatttg tctcagtaa cactccaacc aaaacacaag gtcttggtgc tggcaaagct	300

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gctgatctta cttactggga gagtgctgct cggatgatcg ctgatgtctc caaatctagc 360
aaaatcgttg ttgagaaatc cacggttcct gtgaggacag cagaggctat tgaaaagata 420
ctgacacata acagcaaagg catagagttt cagattctct ctaaccctga atttcttgct 480
gagggactg caattaagga tctttataac ccagaccgtg tgttgattgg tggtagggat 540
actgcagcag ggcaaaaggc tattaagct ttaagagatg tttatgctca ttgggttcca 600
gtggaacaaa tcatttgac gaacctgtgg tccgctgagc tctctaagct tgcagcaaat 660
gcattcttag ctgagaggat atcatctgtc aatgccatgt cagctctatg tgaggcaact 720
ggcgtgatg ttacacaagt tgcgcatgcc gtgggtacag atactagaat tgggtccaaag 780
ttcttgaatg ctagtgttg ttttgggtgga tcatgtttcc aaaaggacat cctaaatctt 840
atctatattt gtgaatgcaa cggcttgccc gaagcagcta attactggaa acaagtcgta 900
aaggtgaacg actatcagaa aatacggttt gcaaaccggg ttgtttcttc aatgtttaac 960
acagtctcgg gcaagaaaat cgcgatcctc ggttttgctt tcaagaagga cacaggtgac 1020
acgagagaga ctccagcgat tgatgtttgt aacagattag ttgcagacaa ggccaagctg 1080
agcatatacg acccacaagt tcttgaagaa cagatcagaa gagatctttc catggctagg 1140
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gtgaatgtcg tctcagatgc ttacgaggca actaaagatg cgcacggcct atgtgtctta 1260
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ccagcttttg tgttcgatgg taggaatggt gttgatgcag tgaagctgcg tgagatcggg 1380
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gcatga 1446

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<210> SEQ ID NO 22

<211> LENGTH: 481

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 22

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Met Val Lys Ile Cys Cys Ile Gly Ala Gly Tyr Val Gly Gly Pro Thr
1           5           10           15
Met Ala Val Met Ala Leu Lys Cys Pro Glu Ile Glu Val Val Val Val
20           25           30
Asp Ile Ser Glu Pro Arg Ile Asn Ala Trp Asn Ser Asp Arg Leu Pro
35           40           45
Ile Tyr Glu Pro Gly Leu Glu Asp Val Val Lys Gln Cys Arg Gly Lys
50           55           60
Asn Leu Phe Phe Ser Thr Asp Val Glu Lys His Val Phe Glu Ser Asp
65           70           75           80
Ile Val Phe Val Ser Val Asn Thr Pro Thr Lys Thr Gln Gly Leu Gly
85           90           95
Ala Gly Lys Ala Ala Asp Leu Thr Tyr Trp Glu Ser Ala Ala Arg Met
100          105          110
Ile Ala Asp Val Ser Lys Ser Ser Lys Ile Val Val Glu Lys Ser Thr
115          120          125
Val Pro Val Arg Thr Ala Glu Ala Ile Glu Lys Ile Leu Thr His Asn
130          135          140
Ser Lys Gly Ile Glu Phe Gln Ile Leu Ser Asn Pro Glu Phe Leu Ala
145          150          155          160
Glu Gly Thr Ala Ile Lys Asp Leu Tyr Asn Pro Asp Arg Val Leu Ile
165          170          175

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-continued

Gly Gly Arg Asp Thr Ala Ala Gly Gln Lys Ala Ile Lys Ala Leu Arg
 180 185 190
 Asp Val Tyr Ala His Trp Val Pro Val Glu Gln Ile Ile Cys Thr Asn
 195 200 205
 Leu Trp Ser Ala Glu Leu Ser Lys Leu Ala Ala Asn Ala Phe Leu Ala
 210 215 220
 Gln Arg Ile Ser Ser Val Asn Ala Met Ser Ala Leu Cys Glu Ala Thr
 225 230 235 240
 Gly Ala Asp Val Thr Gln Val Ala His Ala Val Gly Thr Asp Thr Arg
 245 250 255
 Ile Gly Pro Lys Phe Leu Asn Ala Ser Val Gly Phe Gly Gly Ser Cys
 260 265 270
 Phe Gln Lys Asp Ile Leu Asn Leu Ile Tyr Ile Cys Glu Cys Asn Gly
 275 280 285
 Leu Pro Glu Ala Ala Asn Tyr Trp Lys Gln Val Val Lys Val Asn Asp
 290 295 300
 Tyr Gln Lys Ile Arg Phe Ala Asn Arg Val Val Ser Ser Met Phe Asn
 305 310 315 320
 Thr Val Ser Gly Lys Lys Ile Ala Ile Leu Gly Phe Ala Phe Lys Lys
 325 330 335
 Asp Thr Gly Asp Thr Arg Glu Thr Pro Ala Ile Asp Val Cys Asn Arg
 340 345 350
 Leu Val Ala Asp Lys Ala Lys Leu Ser Ile Tyr Asp Pro Gln Val Leu
 355 360 365
 Glu Glu Gln Ile Arg Arg Asp Leu Ser Met Ala Arg Phe Asp Trp Asp
 370 375 380
 His Pro Val Pro Leu Gln Gln Ile Lys Ala Glu Gly Ile Ser Glu Gln
 385 390 395 400
 Val Asn Val Val Ser Asp Ala Tyr Glu Ala Thr Lys Asp Ala His Gly
 405 410 415
 Leu Cys Val Leu Thr Glu Trp Asp Glu Phe Lys Ser Leu Asp Phe Lys
 420 425 430
 Lys Ile Phe Asp Asn Met Gln Lys Pro Ala Phe Val Phe Asp Gly Arg
 435 440 445
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 450 455 460
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 465 470 475 480
 Ala

<210> SEQ ID NO 23
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: The sequence of designed polynucleotide
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 <400> SEQUENCE: 23

gggaattcgt gaagatctgt tgtattggag ct

32

<210> SEQ ID NO 24
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
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described in Example 1

<400> SEQUENCE: 24

cagaagcttt tagacaaagg caggcatgct ctt 33

<210> SEQ ID NO 25
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
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<400> SEQUENCE: 25

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<210> SEQ ID NO 26
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
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<400> SEQUENCE: 26

aactgcagtt aggcaacggc aggcatgtcc t 31

<210> SEQ ID NO 27
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 1

<400> SEQUENCE: 27

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<210> SEQ ID NO 28
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 1

<400> SEQUENCE: 28

aactgcagtt aggcaagagc aggcatgtcc t 31

<210> SEQ ID NO 29
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 1

<400> SEQUENCE: 29

ggaattcgtg aagatatgct gcataggagc 30

<210> SEQ ID NO 30
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 1

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<400> SEQUENCE: 30
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<210> SEQ ID NO 31
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 2

<400> SEQUENCE: 31
ggaattctca cgaatcgcag tcggttggtg 30

<210> SEQ ID NO 32
<211> LENGTH: 32
<212> TYPE: DNA
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described in Example 2

<400> SEQUENCE: 32
gactgcagtt agttatcgcc atatacatca cg 32

<210> SEQ ID NO 33
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 3

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<210> SEQ ID NO 34
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 3

<400> SEQUENCE: 34
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<210> SEQ ID NO 35
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 4

<400> SEQUENCE: 35
tctgtacgat gcaactacca atgctcagt 29

<210> SEQ ID NO 36
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: The sequence of designed polynucleotide
described in Example 4

<400> SEQUENCE: 36

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tatcttacct ggggtcaaatg acgaacataa 30

<210> SEQ ID NO 37
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 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: The sequence of designed polynucleotide
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<400> SEQUENCE: 37

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<210> SEQ ID NO 38
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 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: The sequence of designed polynucleotide
 described in Example 4

<400> SEQUENCE: 38

aactgcagtt aaaaggtggt cacggatttt gcaagattc 39

<210> SEQ ID NO 39
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 <212> TYPE: DNA
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 <220> FEATURE:
 <223> OTHER INFORMATION: The sequence of designed polynucleotide
 described in Example 1

<400> SEQUENCE: 39

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<210> SEQ ID NO 40
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
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<210> SEQ ID NO 41
 <211> LENGTH: 30
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<210> SEQ ID NO 42
 <211> LENGTH: 30
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: The sequence of designed polynucleotide
 described in Example 6

<400> SEQUENCE: 42

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<210> SEQ ID NO 43
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: The sequence of designed polynucleotide
 described in Example 13

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33

<210> SEQ ID NO 44
 <211> LENGTH: 35
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: The sequence of designed polynucleotide
 described in Example 13

<400> SEQUENCE: 44

ccgagctctt agttatcgcc atatacatca cgtgt

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The invention claimed is:

1. A method of producing hyaluronic acid, comprising co-expressing a protein with hyaluronic acid synthase activity, an exogenous protein with glutamine:fructose-6-phosphate amidotransferase activity, and an exogenous protein with uridin-5'-diphospho(UDP)-glucose dehydrogenase activity in a plant cell or a plant.

2. A method of producing hyaluronic acid, containing the steps of:

- (1) transforming a plant cell or a plant using a recombinant expression vector, the recombinant expression vector having DNA encoding a protein with hyaluronic acid synthase activity, DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity, and DNA encoding a protein with uridin-5'-diphospho(UDP)-glucose dehydrogenase activity, each said DNA being under control of a plant promoter;
- (2) growing a transformant obtained by the transformation; and
- (3) isolating hyaluronic acid produced by the transformant.

3. The method of producing hyaluronic acid according to claim 2, wherein the promoter is an organ-specific or a tissue-specific promoter.

4. The method of producing hyaluronic acid according to claim 2, wherein the DNA encoding a protein with hyaluronic acid synthase activity is DNA of (a) or (b) below:

- (a) DNA having a nucleotide sequence represented by SEQ ID NO: 1 or 3; or
- (b) DNA complementarily hybridizing to the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

5. The method of producing hyaluronic acid according to claim 1, wherein the protein with hyaluronic acid synthase activity is a protein of (a) or (b):

- (a) a protein having an amino acid sequence shown by SEQ ID NO: 2 or 4; or
- (b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

6. The method of producing hyaluronic acid according to claim 2, wherein DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity and DNA

25 encoding a protein with UDP-glucose dehydrogenase activity are DNA derived from chlorella virus or *Arabidopsis*.

7. The method of producing hyaluronic acid according to claim 2, wherein DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity is DNA of (a) or (b) below:

- (a) DNA having a nucleotide sequence shown by SEQ ID NO: 5, 7 or 9; or
- (b) DNA complementarily hybridizing to the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

8. The method of producing hyaluronic acid according to claim 1, wherein the protein with glutamine:fructose-6-phosphate amidotransferase activity is a protein of (a) or (b) below:

- (a) a protein having an amino acid sequence shown by SEQ ID NO: 6, 8 or 10; or
- (b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

9. The method of producing hyaluronic acid according to claim 2, wherein DNA encoding a protein with UDP-glucose dehydrogenase activity is DNA of (a) or (b) below:

- (a) DNA having a nucleotide sequence shown by SEQ ID NO: 11, 13, 17, 19, or 21; or
- (b) DNA complementarily hybridizing to the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

10. The method of producing hyaluronic acid according to claim 1, wherein the protein with UDP-glucose dehydrogenase activity is a protein of (a) or (b) below:

- (a) a protein having an amino acid sequence shown by SEQ ID NO: 12, 14, 16, 18, 20 or 22; or
- (b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

11. The method of producing hyaluronic acid according to claim 1, wherein the plant is selected from the group consisting of angiosperms, gymnosperms, pteridophytes and bryophytes.

12. The method of producing hyaluronic acid according to claim 3, wherein one or more organs are selected from the

group consisting of roots, stems, stem tubers, leave, floral organs, tuberous roots, seeds and shoot apices.

13. The method of producing hyaluronic acid according to claim 3, wherein one or more tissues are selected from the group consisting of epidermis, phloem, soft tissues, xylem and vascular bundles.

14. A transgenic plant cell or a transgenic plant or a progeny, organ or tissue thereof having an ability to produce hyaluronic acid by co-expressing a protein with hyaluronic acid synthase activity, an exogenous protein with glutamine:fructose-6-phosphate amidotransferase activity, and an exogenous protein with uridin-5'-diphospho(UDP)-glucose dehydrogenase activity.

15. A transgenic plant cell or a transgenic plant transformed with a recombinant expression or a progeny, organ or tissue thereof vector containing DNA encoding a protein with hyaluronic acid synthase activity, DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity, and DNA encoding a protein with uridin-5'-diphospho(UDP)-glucose dehydrogenase activity, each said DNA being under control of a plant promoter.

16. The transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to claim 15, wherein the promoter is an organ-specific or a tissue-specific promoter.

17. The transgenic plant cell or the transgenic plant, the progeny thereof, or the organ or the tissue thereof according to claim 15, wherein the DNA encoding a protein with hyaluronic acid synthase activity is DNA of (a) or (b) below:

(a) DNA having a nucleotide sequence shown in SEQ ID NO: 1 or 3; or

(b) DNA complementarily hybridizing to the DNA having the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

18. The transgenic plant cell or the transgenic plant according to claim 14, wherein the protein with hyaluronic acid synthase activity is a protein of (a) or (b) below:

(a) a protein having an amino acid sequence shown by SEQ ID NO: 2 or 4; or

(b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

19. The transgenic plant cell or the transgenic plant, the progeny thereof, or the organ or the tissue thereof according to claim 15, wherein the DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity and the DNA encoding a protein with UDP-glucose dehydrogenase activity are derived from chlorella virus, *Arabidopsis*, or chlorella virus and *Arabidopsis*.

20. The transgenic plant cell or the transgenic plant, the progeny thereof; or the organ or the tissue thereof according to claim 15, wherein the DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity is DNA of (a) or (b) below:

(a) DNA having a nucleotide sequence shown by SEQ ID NO: 5, 7 or 9; or

(b) DNA complementarily hybridizing to the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

21. The transgenic plant cell or the transgenic plant, the progeny thereof, or the organ or the tissue thereof according to claim 14, wherein the protein with glutamine:fructose-6-phosphate amidotransferase activity is a protein of (a) or (b) below:

(a) a protein having an amino acid sequence shown by SEQ ID NO: 6, 8 or 10; or

(b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

22. The transgenic plant cell or the transgenic plant, the progeny thereof, or the organ or the tissue thereof according to claim 15, wherein DNA encoding a protein with UDP-glucose dehydrogenase activity is DNA of (a) or (b) below:

(a) DNA having a nucleotide sequence shown by SEQ ID NO: 11, 13, 15, 17, 19, or 21; or

(b) DNA complementarily hybridizing to the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

23. The transgenic plant cell or the transgenic plant, the progeny thereof, or the organ or the tissue thereof according to claim 14, wherein the protein with UDP-glucose dehydrogenase activity is a protein of (a) or (b) below:

(a) a protein having an amino acid sequence shown by SEQ ID NO: 12, 14, 16, 18, 20 or 22; or

(b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

24. The transgenic plant cell or the transgenic plant, the progeny thereof, or the organ or the tissue thereof according to claim 14, wherein the plant is selected from the group consisting of gymnosperms, gymnosperms, pteridophytes and bryophytes.

25. The transgenic plant cell or the transgenic plant, the progeny thereof; or the organ or the tissue thereof according to claim 14, wherein the organ is one or more organs selected from the group consisting of roots, stems, stem tubers, leaves, floral organs, tuberous roots, seeds and shoot apices.

26. The transgenic plant cell or the transgenic plant, the progeny thereof, or the organ or the tissue thereof according to claim 14, wherein the tissue is one or more tissues selected from the group consisting of epidermis, phloem, soft tissues, xylem and vascular bundles.

27. Plant extract obtained from the transgenic plant cell or the transgenic plant, the progeny having the same nature thereof, or the organ or the tissue thereof according to claim 14, wherein the plant extract has the ability to produce hyaluronic acid by co-expressing a protein with hyaluronic acid synthase activity, an exogenous protein with glutamine:fructose-6-phosphate amidotransferase activity, and an exogenous protein with uridin-5'-diphosphate(UDP)-glucose dehydrogenase activity.

28. The plant extract according to claim 27, wherein the plant extract contains hyaluronic acid.

29. A recombinant expression vector comprising DNA encoding a protein with hyaluronic acid synthase activity, DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity, and DNA encoding a protein with uridin-5'-diphospho(UDP)-glucose dehydrogenase activity, each said DNA being under control of a plant promoter.

30. The recombinant expression vector according to claim 29, wherein the promoter is an organ-specific or a tissue-specific promoter.

31. The recombinant expression vector according to claim 29, wherein the DNA encoding a protein with hyaluronic acid synthase activity is DNA of (a) or (b) below:

(a) DNA having a nucleotide sequence shown in SEQ ID NO: 1 or 3; or

(b) DNA complementarily hybridizing the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

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32. The recombinant expression vector according to claim 29, wherein the protein with hyaluronic acid synthase activity is a protein of (a) or (b) below:

- (a) a protein having an amino acid sequence shown by SEQ ID NO: 2 or 4; or
- (b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

33. The recombinant expression vector according to claim 29, wherein the DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity and the DNA encoding a protein with UDP-glucose dehydrogenase activity are DNA derived from chlorella virus, *Arabidopsis*, or chlorella virus and *Arabidopsis*.

34. The recombinant expression vector according to claim 29, wherein the DNA encoding a protein with glutamine:fructose-6-phosphate amidotransferase activity is DNA of (a) or (b) below:

- (a) DNA having a nucleotide sequence shown by SEQ ID NO: 5, 7 or 9; or
- (b) DNA complementarily hybridizing to the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

35. The recombinant expression vector according to claim 29, wherein the protein with glutamine:fructose-6-phosphate amidotransferase activity is a protein of (a) or (b) below:

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(a) a protein having an amino acid sequence shown by SEQ ID NO: 6, 8 or 10; or

(b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

36. The recombinant expression vector according to claim 29, wherein the DNA encoding a protein with UDP-glucose dehydrogenase activity is DNA of (a) or (b) below:

(a) DNA having a nucleotide sequence shown by SEQ ID NO: 11, 13, 15, 17, 19, or 21; or

(b) DNA complementarily hybridizing to the nucleotide sequence of (a) under highly stringent conditions in which washing is performed in salt concentrations equivalent to that of 0.1×SSC or 0.1% SDS at 60° C.

37. The recombinant expression vector according to claim 29, wherein the protein UDP-glucose dehydrogenase activity is a protein of (a) or (b) below:

(a) a protein having an amino acid sequence shown by SEQ ID NO: 12, 14, 16, 18, 20 or 22; or

(b) a protein having the amino acid sequence of (a) with one to ten amino acids deleted, substituted or added.

38. A method of generating a transgenic plant cell or the transgenic plant comprising transforming a plant cell or a plant using a vector according to claim 29, wherein the transgenic plant cell or the transgenic plant produces hyaluronic acid.

* * * * *